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HOW PARASITES TOLERATE THEIR HOSTS[1]

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A graduate student was asked the following question in a predoctoral examination: How do parasites maintain themselves in their hosts? Her answer was mostly a well-organized discussion of the structural and physiological adaptations of the parasitic worms for living inside their hosts. Under the former she noted the sensory organs, nervous system, and musculature of parasites that somehow produce the coordinated movements that bring about elective localization in the body of the host, and the accessory holding devices such as suckers, bothria, hooks, spines, holdfast organs, adhesive disks, buccal capsules and proboscides variously equipped. The predominant development of ovaries, testes and secondary sexual organs to insure the statistical advantages of a plenitude of posterity did not escape her attention. Under physiology, lip service was paid to that vague term "compatibility of protoplasms of host and parasite" about which so little is known. There followed a discussion of how parasites have become regulated to the amount of oxygen available to them in their particular habitats in the host body by utilizing anaerobic processes to varying degrees, and of matters relating to nutrition, excretion and secretion.

Parasites do maintain themselves in their hosts to a considerable extent through adaptations of structure, physiology and life cycle. But assuming, firstly, that a particular parasite is admirably equipped with functional mechanical devices for keeping to its wonted place in the host's body, and, secondly, that its fundamental processes are basically salubrious—i.e., it could eat, digest, eliminate, respire, assimilate, grow and reproduce well in its environment if given the chance—there is still a third requirement that it must meet in order to maintain itself inside the host. It is an unwelcomed invader in hostile territory, and as such has constantly to contend with enzymes, antibodies, hormones, foreign proteins, protein-split products, tissue defense reactions and predatory phagocytes. How is the parasite equipped to cope with these factors in its environment? Very much has been written that might be included under such a subject as *How Hosts Tolerate Their Parasites,* but not so much has been said about *How Parasites Tolerate Their Hosts.* In developing the latter subject, it may be necessary to step from the sure ground of demonstrated scientific fact over onto the hazardous quicksands of speculation for part of the way. But who would deny the moderate use of imagination and intrepidity to the worker in quest of the solution to a complex natural phenomenon?

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While engaged in the routines of drug testing (Becker, 1949), we noted the disappearances and reappearances of parasites in the blood of ducks which had survived the primary attack of lophurae malaria (Becker, Brodine and Clappison, 1949). More frequently, we observed minor recessions and advances in parasite level during intervals of patency. On occasions, there were major parasite recrudescences to which the host either succumbed or built up resistance which reduced the parasitemia to innocuous proportions, at least for a time. When it became possible for us to investigate the immune state in lophurae malaria of ducks, the first problem that presented itself was to test for protective antibodies in the circulating blood of so-called recovered ducks by injecting the plasma into ducklings inoculated with parasitized duck erythrocytes. The initial experiment was to be patterned in a general way after the one conducted by the Taliaferros (1940) demonstrating protective antibodies in the serum of recovered chicks.

Owing to a certain impatience to get the project started and to the circumstance that very young ducklings were not immediately available, chicks were inoculated, intravenously, with parasitized duck erythrocytes. Fifteen 10-day-old chicks received the first of five daily injections of plasma from so-called recovered ducks about an hour before the parasites were injected, and 15 control chicks of the same hatching received physiological salt solution in lieu of plasma. No parasites could be seen in the stained smears of any of the chicks after 24, 48, or 72 hours, because the inoculative dosage of the parasite was too light, considerably lighter than that subsequently employed. Parasites commenced to appear in the blood of nine of the 15 recipients of duck plasma on the fourth, fifth or sixth days, and the maximum parasitemias varied from 0.5 to 3.0 percent. The parasitemia attained 1.5 percent in the only control with microscopically demonstrable parasites.

Nine of 15 chick recipients of immune duck plasma with parasitemia, compared with one of 15 chick recipients of salt solution with parasitemia! A startling and paradoxical, not to say frustrating, outcome! This particular experiment has been neither repeated nor published up to the present time, but after thinking it over for several months we returned to experimenting with duck plasma or serum in chicks injected with parasitized duck cells, using henceforth larger doses of the parasitized duck cells. In due time, it was proved conclusively that when both duck plasma (either normal or from recovered ducks) and parasitized duck erythrocytes were introduced into the blood stream of young chicks, the duck plasma somehow or other exerted a protective influence over the duck cells, so that many of these cells were spared for a time from the chick's innate destructive forces aimed at foreign bodies in its circulation (Becker, Brodine, Marousek, and Byrd, 1949; Becker, Schwink, Byrd and Conn, 1950). This, the sparing phenomenon, was produced in the body of the living chick.

The translation of the sparing phenomenon into terms of well-known immunological phenomena was realized to a certain extent. Many, but not all, chicken plasmas were found to possess the in vitro property of agglutinating duck erythrocytes, whether parasitized or nonparasitized (Becker, Schwink and Prather, 1951). Chicken plasmas which had been frozen long or often enough generally agglutinated better than fresh plasmas. (See also Schwink and Becker, 1951.) Heating at 56° C for 30 minutes also enhanced their respective agglutinating properties to about the same degree. It was learned that duck plasma, often in great dilution,

possessed the property of hemaglutinin-inhibition in the test tube. In other words, it spared the duck erythrocytes from agglutination by frozen or heated chicken plasmas. Most fresh, unfrozen and unheated chicken plasmas likewise agglutinated duck cells, and most of them in turn were inhibited by duck plasma, but in about a fifth of them the potency was actually enhanced. A few of the fresh plasmas seemed to be unaffected by duck plasma. It is altogether possible that the variable in vitro behavior of the hemagglutinin in different fresh chicken plasmas treated with duck plasma provides a cue to the great variability of results obtained in the in vivo experiments which established the authenticity of the sparing phenomenon (Becker, Brodine, Marousek and Byrd, 1949; Becker, Schwink, Byrd and Conn, 1950). It should be kept in mind that antibody of a certain type may act either as an opsonin or an agglutinin.

Were sparing activity, which occurred in the animal, and hemagglutinin-inhibition, which occurred in vitro, but counterparts of one and the same phenomenon? Obviously, one approach to the solution of the problem was the separation of a substance from duck plasma or from duck organs that would reproduce one or the other, or both effects. Such a substance was actually separated from duck plasma, partly as the result of a suggestion by an interested person and partly by chance. The first break came when an alcoholic extract of duck plasma was found to manifest both hemagglutinin-inhibition and sparing activity (Becker and Schwink, 1951). Of even greater importance, it was found that part of the coagulated plasma which remained after treatment with strong ethyl alcohol or alcohol-ether was soluble in physiological salt solution and that the solution inhibited hemagglutination. Later, seromucoid separated from duck plasma and an alcoholic extract of duck liver were found to be capable of reproducing both sparing activity and hemagglutinin-inhibition (Becker, Schwink and Probst, 1952; Becker and Schwink, 1953). Here, then, was proof of the etiological oneness of the two phenomena.

But what is a seromucoid? I can only explain that it is a complex component of plasma belonging to a class of organic compounds called mucoids or mucoproteins, and that is consists of a complex sugary compound built up from amino sugars, such as hexoseamine, and simple hexoses, polymerized to a considerable degree and in combination with peptides, and probably also with lipids, such as sterols. It is sometimes called protein-sugar. Its concentration in human plasma has been shown to increase under certain pathological conditions, as in cancer, pneumonia and tuberculosis. It is quite thermostable, and is not precipitated by brief boiling. It is precipitated, however, with strong ethyl alcohol without affecting its subsequent solubility in physiological salt solution. Like other proteins, it is precipitated with various strengths of sodium sulphate or ammonium sulphate solutions, and it reacts positively, though not strongly, in the xanthoproteic, biuret and ninhydrin tests. It gives a positive reaction with the Molisch and the Benedict's tests for sugar, and a stronger reaction after hydrolysis with acid and heat. In hemagglutinin-inhibition tests duck seromucoid showed persistent reactivity with the agglutinating chicken plasma, i.e., its potency lasted over a period of time, for there was more inhibition after 48-hour contact than after 24-hour contact, more than 24-hour contact than after two-hour contact, more after two-hour contact than after 30-minute contact, and more after 30-minute contact than after four-minute contact (Becker, Schwink and Probst, 1952). (In all these cases, the duck erythrocytes were added at the

end of the reaction period.) This, then, is the material that buffered the onslaught of the bodily defenses of the chick on the parasitized duck erythrocytes commingled with the chick's own blood cells. We believe, at present, that the only role the parasite played in the phenomenon was serving as the label for the duck erythrocyte, but there is no positive proof of it.

Meyer (1945) stated that many mucopolysaccharides and mucoids are quite effective inhibitors of proteolytic enzymes, and that the mucopolysaccharide component of certain mucoids possesses what is called blood group A activity, which can be demonstrated by inhibition of either agglutination of human A erythrocytes by anti-A serum or hemolysis of sheep erythrocytes by anti-A rabbit serum. Mucins and mucoids have important functions in the vertebrate body (Cf. Burnet, 1948). According to certain authorities (Cf. Maximow and Bloom, 1942) gastric mucus forms on the mucous membrane of the stomach a layer which has an inhibiting effect on the action of gastric juice, thus preventing autodigestion of the stomach lining. The mucus takes the punishment, vicariously. (Others, however, have postulated an anti-enzyme in this role.) Ovomucoid, from egg white, inhibits trypsin (Frankel-Conrat *et coll.*, 1949), the acid groups of the former combining directly with the amino groups of the enzyme. Since ovomucoid does not inhibit agglutination of duck cells by chicken plasma (unpublished) and, as Becker, Schwink, Byrd and Conn (1950) have found, egg white does not reproduce the sparing phenomenon, trypsin cannot be involved in either reaction.

From the time the sparing action of duck plasma was first observed we have labored with the conviction that the phenomenon holds the secret to relapse in malaria. Unfortunately for our conviction, several attempts to produce relapses in recovered chicks and ducks by intravenous injection of duck seromucoid resulted in failure (unpublished). Seromucoid, however, is probably not a single and homogeneous subtsance, for Winzler *et coll.* (1948) found in their earlier experiments that a preparation of seromucoid from human plasma consisted of three electrophoretically demonstrable components. Also, Rimington (1940) made fractional precipitations of purified ox seromucoid by adding alcohol in successive stages and removing each fraction as formed. His analyses show that the ratio of carbohydrate to protein in the precipitates became considerably higher as the strength of the alcohol was increased. Perhaps either comparatively carbohydrate-rich or lipid-rich fractions are immunologically more active than the other fractions. Rimington could not be sure, however, whether the fractions pre-existed in the serum or resulted from the process of isolation.

So far as polysaccharides are concerned, bacteriologists have long been aware that these substances make up most of the capsular material of pathogenic bacteria, although it is possible that nitrogenous compounds may also be present in combination with them. The capsules of pathogenic bacteria seem not to be primarily for aggressive warfare, but rather for defense against phagocytes. Encapsulated bacteria ingested by leucocytes often resist the digestive enzymes within the phagocytic cell and in due time escape unharmed. One of the most virulent microorganisms in human disease, *Pneumococcus* Type III, is said to possess a particularly large capsule and, in its highly virulent strains, a wide slimy extension of the capsule which prevents phagocytic cells from ingesting it (Wood and Smith, 1949). It is not known to what extent the mucoid materials of capsules are bound to protein, but

it is certain that they possess antigenic specificity, and that when antibodies to their capsules are produced the bacteria can no longer tolerate their host.

Stacy (1953) comments about bacterial polysaccharides as follows: "In pathogenic micro-organisms the capsule acts as a first line of defense of the cell against attack of phagocytes, and it appears to combine immediately with homologous immune proteins or antibodies."

One may ask, to what extent are animal parasites possessed of protective polysaccharide compounds, which might romantically be called their magic chemical armor? Canning (1929) separated the cuticle from the underlying tissues of the nematode *Ascaris*, then dried and powdered it, and suspended the powder in saline before injecting it into rabbits. The high titers obtained in precipitin tests using immune serum of the injected rabbits and cuticle antigen indicated the potent immunizing property of the cuticle. The anti-cuticle serum, however, lacked species specificity, for it reacted equally well with the cuticles of certain other ascarids. Thus it was shown that a worm's cuticle possessed chemical activity, and the possibility became evident that it could serve as more than a mechanical barrier between the parasite's tissues and its environment.

Oliver-González (1943) confirmed and extended the findings of Canning, and noted the effect of antisera on the living larvae, but the sera seemed to have no direct effect upon the cuticle, though they did affect materials seeping through from underneath. Later (1944b, 1946b), he demonstrated blood group A and blood group B activity (i.e., removal or reduction of agglutinins for these two blood cell substances) of polysaccharides isolated from *Ascaris* and of *Ascaris* cuticle, also of polysaccharides from *Ascaris lumbricoides, Ascaris suum, Necator americanus, Trichinella spiralis, Fasciola hepatica, Schistosoma mansoni, Taenia saginata* and *Cysticercus cellulosae*, when added to human serum containing the related isoagglutinins. Worm polysaccharide was considered to be an antigenic substance, common to A and B blood groups, whose serological specificity depended on the protein group attached to it (Oliver-González and Torregrosa, 1944; Oliver-González, 1946a).

Oliver-González and González (1949) noted complete absorption of a_2 isoagglutinin and partial absorption of a_1 isoagglutinin when *Trichinella spiralis* larvae and *Schistosoma mansoni* adults were incubated in human B serum, but there was no absorption of β isoagglutinin in human A serum. The polysaccharide content of the worms was reduced during incubation in human B serum, and the circumstances suggested that the polysaccharide lost by the worms absorbed the agglutinin directly. At this point Oliver-González and González ventured the following astute speculation: "The release of the polysaccharide by the infective organism upon its penetration into the host may be essential for its survival."

It has already been mentioned that the cuticle of ascarids possesses strong antigenic properties when removed, powdered, dissolved and injected into rabbits. Thus, it is not an inert layer, although Oliver-González considers it too insoluble to evoke demonstrable antibodies while the worm is living in the host. Kruidenier (unpublished), according to Baer (1951), has found by chemical analyses that the various layers of the cuticle of *Ascaris* are combinations of mucroprotein; likewise, the cuticle of the fluke *Paragonimus*. In both of these parasites the mucroproteins are probably considerably polymerized. On the basis of Kruidenier's studies on

the cuticles of nematodes and flukes, Baer (1951) postulated that the cuticle of cestodes is also of mucoprotein nature. Kent (1947) found in the tapeworm *Moniezia expansa* two compounds of protein with glycogen which he called baerine and moniezine. Baer remarked that the mucopolysaccharide nature of the cuticula of nematodes, cestodes and trematodes may explain how they escape digestion in the host. Bushnell and Erwin (1949a, 1949b) extracted a powerful trypsin inhibitor from *Ascaridia,* but they did not identify it with any particular class of chemical compounds. Baer quotes de Waele to the effect that living segments of *Taenia saginata* immersed in artificial digestive fluid resist the enzymes, but digestion proceeds almost immediately when the cuticle is cut or injured. Perhaps, then, mucopolysaccharides or mucoproteins are the real antienzymes! They exhibit considerably more thermostability, however, than one might expect of so-called antienzymes.

In developing the subject of how parasites tolerate their hosts, there is a special pertinency in the work of Kruidenier (1951a) concerning penetration of the tissues of the intermediate host by virgulate cercariae. According to him, the virgula is a gland of amorphous appearance contained within the oral sucker of certain of the xiphidiocercariae and opening by pores on the dorsal surface. Mucicarmine and certain other dyes stain it and the mucoid glands further back in the body in a manner characteristic of the mucoproteins. These glands appear late in the development of the cercariae and seem to disappear after penetration. Their adhesive content appears to be used during penetration of another host, such as an insect. Penetrating larvae become enveloped in the discharges of the mucoid virgular substance as they migrate in the host.

Kruidenier comments that the secretion may provide constant lubrication valuable in easing cercarial movements through the tissues, defense against antagonistic fluids of the host tissue reactions and, on account of the fact that mucoids frequently show high resistance to enzymatic digestive activity, protection to the penetrating cercaria against the secretion of its own penetration glands. The same author (1951b, 1952, 1953) has also investigated the distribution and role of mucoid materials in other kinds of cercariae. Considerable credit goes to Kruidenier for his keenness in connecting his observations on cercarial development with the utility of a substance formed and spent during the lives of certain cercariae.

We have been discussing mostly the role of protein-sugars in the defense of the parasite; but let it not be overlooked that the shoe may well fit the other foot also, for they seem to function in the defense of the host against the parasite, I am thinking, of course, of the notable contributions on the subject of mucus in age resistance of chickens to the nematode *Ascaridia galli* which were discussed before this society twelve years ago in the presidential address of Dr. J. E. Ackert (1942). Let us review some of the salient facts that were brought out (see also Frick and Ackert, 1948). *Ascaridia galli,* a parasite of the anterior part of the small intestine, finds conditions more suitable for development in younger chickens than in older chickens. Increase in resistance as the birds grow older is *pari passu* with significant increase in density of goblet (mucous) cells in the duodenal epithelium up to the age of about 124 days and, hence, with the amount of mucus secreted.

In vitro, autoclaved duodenal mucus commences to inhibit the growth of *Ascaridia* when the chicken donor reaches the age of 54 days or so, and its potency increases as the chick ages up to about 125 days. Although the density of goblet

cells increases little after the latter age, the growth-retarding quality of the mucus still tends to increase as the chicken grows older. Duodenal mucus extracts of adult hogs and dogs cause the early death of *Ascaridia* exposed to them in vitro (Frick and Ackert, 1941). As was suggested, such effects may help explain the host-specificity of the worm. The factor in mucus responsible for growth-inhibition is unknown, but it has been found to be both soluble in aqueous salt solution and thermostable (hence, it is not an antibody). It may turn out to be mucin itself, a glycoprotein. The observed increase in potency of mucus from older chickens does not nullify this possibility, because chemical change in the molecules of mucin, such as increase of either the polysaccharide or lipid component, for example, could conceivably be responsible.

The presence of blood group A_2 substance in human malarial parasites and 10 species of parasitic bacteria was postulated by J. Oliver-González and L. M. González (1949) after they had observed that these microorganisms absorbed a_2 agglutinin from the serum of group B individuals. Oliver-González (1944a) had previously demonstrated an increase in a and β agglutinin titers in the serum of patients having had repeated attacks of malaria and an increase of a_2 agglutinin titer in the serum of patients with blackwater fever. He was led to the deduction that the malarial parasite may be the source of the agglutinogen (A_2) which immunizes the host so that increase of a_2 agglutinin results. When this happens, autoagglutination of A_2 cells and weaker agglutination of A_1 and B cells follow. In this way arise the intravascular agglutination of erythrocytes, characteristic of blackwater fever and observed also in certain cases of malaria, and the subsequent intravascular hemolysis characteristic of blackwater fever, a condition so often associated with human malaria in the tropics. (See also Malamos, 1937, and Lack, 1942.)

While the comments of Oliver-González (1944a) and the account of the sparing phenomenon are still fresh in mind, the speaker would like to ask your permission to present a hypothesis of the mechanism of relapse in malaria. First of all, let us conceive of a normal higher organism, man or duck, as being both at peace and at war with itself. (One author prefers to regard man as a "self-buffering, automatic thermostat.") Normal erythrocytes are coursing through his body inside the blood channels. The red bone marrow is constantly contributing newly formed erythrocytes to the blood stream as the damaged or otherwise altered corpuscles are eliminated from the circulation by both hemolysis and ingestion by the predatory cells known as macrophages. (The present emphasis will be placed on phagocytosis rather than hemolysis, because more seems to be known about how it operates in the living organism.) In the plasma circulates an auto-antibody, opsonin—whose production is stimulated by the altered cells—that "butters" (as George Bernard Shaw put it in *The Doctor's Dilemma*) the superannuated erythrocytes for the predatory macrophages. Another component of the plasma is seromucoid, a mucoprotein, existing in loose combination with opsonin in the presence of normal erythrocytes, thus protecting them from destruction and the host from anemia. The opsonin, however, is drawn competitively from the combination into union with the chemically altered cells, rendering them attractive to the phagocytes. Drawing the line between normal cells and altered cells is no doubt a delicate matter regu-

lated by the qualities of both seromucoid and opsonin, which in turn reflect certain innate constitutional characteristics of the host.

Malarial parasites introduced into the blood stream of the host commingle with the normal erythrocytes, and invade some of them. Most of the parasitized cells, now in the category of altered cells, become attractive to the opsonin and, as L. G. Taliaferro (1925) and E. Hartman (1927) have shown, are eliminated from the circulation in considerable numbers, even in the non-immune host. In the case of *Plasmodium cathemerium* in the normal canary, i.e., the bird without previous experience with malaria, the parasite mortality per generation was calculated to be about 67 per cent! Boyd (1939) arrived at an even higher figure! The rest of these erythrocyte-parasite combinations, like cowbird eggs in the nest of a chipping sparrow, somehow escape detection. They are spared from the opsonin's kiss of death because the opsonin in combination with the seromucoid is not related closely enough to them antigenically. (These statements are made with full knowledge of Gingrich's (1941) objection on experimental grounds to phagocytosis of parasitized erythrocytes in cathemerium malaria as the explanation for L. G. Taliaferro's observation. The speaker cannot concur in Gingrich's conclusions, on the basis of his evidence.) The mucoproteins of these parasite-erythrocyte combinations must indeed be qualitatively very much like those of the normal red cells of whatever blood group. In support of this deduction may be cited the successful experiments of Kligler and Yoeli (1941) who used normal chicken erythrocytes for antigen to fix complement in complement fixation tests for human malaria, and the more successful tests of Heidelberger and Meyer (1944) who used the stromata of normal human erythrocytes. (See also Coffin, 1951a and 1951b, as well as Schwink, 1953.)

But the mucoproteins of normal and parasitized erythrocytes are not quite identical chemically; because the host, if it survives, eventually produces opsonins specifically adapted for combining more with the parasite-erythrocyte combinations and the parasites than with normal erythrocytes. Phagocytosis ensues. And so the host gains, temporarily at least, the advantage over the parasite. Then the parasitemia subsides markedly, sometimes to the point of so-called latency. It should be explained that the discriminating abilities of this newer anti-malarial opsonin are probably not too sharp, because Taliaferro and his collaborators (Cannon and Taliaferro, 1931; Taliaferro and Cannon, 1936; Taliaferro and Mulligan, 1937) have noted phagocytosis of both parasitized and uninfected red cells during the crisis in one of the bird malarias, apparently, and in several monkey malarias. It was suggested, however, that the phagocytosed, presumably normal cells of the monkey might have been injured by toxin. In addition, Zuckerman (1945) has observed that macrophages in hyperimmune serum ingest uninfected red cells as well as the malarial parasites and parasitized cells. (Ben-Harel, 1951, however, found that the normal serum of resistant ducks contained an antibody as effective, in vitro, in promoting phagocytic activity of duck macrophages for duck erythrocytes parasitized with *P. lophurae* as the acquired antibody of hyperimmune serum.) Coffin (1951) has reported that much of the protective effect of immune serum in ducklings injected with *P. lophurae* is attributable to antibodies against the enveloping erythrocyte.

What happens to produce the parasite relapse? In the first place, the specific opsonin for the parasitized cells or the parasites supposedly sinks to a lower level after some time following the crisis, while the parasite continues its asexual multiplication somewhere in the body. (How these surviving parasites escape destruction is another story. It appears that the spectrum of the opsonin, while broad enough to include even certain uninfected cells, may still not have enough range to cover the relatively few surviving parasite-erythrocyte combinations. Or, in case the blood does become free of parasites, as in infection with the St. Elizabeth strain of *Plasmodium vivax,* the seeding of the erythrocytes would necessarily be from exo-erythrocytic forms, as Cooper, Ruhe and Coatney, 1949, realized.) Since in lophurae malaria many recovered ducks actually show seemingly uninterrupted low-grade parasitemias in the periods between the major parasite recrudescences, it would seem that in such cases there is sufficient and constant enough stimulation of the host's defenses by antigen to render it unwarrantable to try to account for relapse solely on the basis of insufficient antibody.

It may be that for some reason or other the host produces qualitatively different seromucoids as it ages, or during different seasons of the year, or under still other environmental conditions, or, even more probably, through a certain randomness of synthesis. Some of them not present at the time the antibody was formed combine with anti-parasite opsonin to the obvious advantage of the parasite, and relapse follows. On the other hand, the surviving parasite itself may produce, through a certain randomness of synthesis, or adaptively, new mucoproteins in very much the same way that certain bacteria produce so-called "adaptive enzymes" for particular substrates. (Let me interpolate here that bacteriologists know much more about "transformations" of types of bacteria, along with bacterial behavior, than protozoologists do about similar phenomena in the protozoa; for example, the demonstration of Avery, MacLeod and McCarty, 1944, that the unencapsulated, attenuated variant of *Pneumococcus* Type II could be transformed into the encapsulated, pathogenic *Pneumococcus* Type III through contact with desoxyribonucleic acid isolated from *Pneumococcus* Type III.) In either case, after the exhaustion of the affinities of the existing anti-parasite opsonin and the resulting parasite relapse, the new parasite mucoprotein would become sufficiently concentrated to be antigenic and stimulate the production of new and related opsonin. The relapse becomes suppressed as the result of the ensuing phagocytosis of parasitized erythrocytes by the macrophages in spleen, liver and bone marrow.

The hypothesis requires two fundamental assumptions, for both of which there is a certain amount of evidence: (1) a certain physiologic mechanism that regulates the composition of the blood's red corpuscle component; (2) the adaptation of this basic mechanism to a special situation, the invasion of the blood stream by an endoerythrocytic parasite. More specific requirements of the hypothesis are, first, chemically or functionally related, but not identical, polysaccharide compounds in the erythrocytes, the parasite-erythrocyte combinations of the primary infection, the parasite-erythrocyte combinations of the relapse, and the seromucoid of the plasma; secondly, the appearance in the blood of a newer polysaccharide configuration that upsets the equilibrium between the host and a parasite in abeyance; and thirdly, an opsonin spectrum with components imperfectly related to the polysaccharide components of the erythrocyte-parasite spectrum.

The hypothesis assumes antigenic properties of the seromucoid and a certain amount of community of antigen in the seromucoid and erythrocyte-parasite combinations. In a preliminary attempt to test this assumption, one group of eight young Hampshire Red chicks was submitted to a course of intramuscular injections with plasma from the normal male duck, and another group of eight from the same hatching to injections with physiological salt solution. Both groups were inoculated with a chicken-passaged strain of *Plasmodium lophurae* four days after the last injection. The courses of the ensuing infections in the two groups were markedly different as the result of the two treatments, in that the plasma-recipients exhibited significantly greater resistance to the increase of the parasite than the controls (Table 1). We have this evidence and some of a slightly different nature for overlapping antigenic qualities of normal duck plasma and the duck erythrocyte combination with *Plasmodium lophurae*. Let me remind you that "dextran," a blood plasma "extender," is a polysaccharide of high molecular weight separated from the capsule of another microorgansm, the free-living bacterium *Leuconostoc mesenteroides*. It can circulate in the blood stream of man for a few days in, ap-

TABLE 1.—*Percentage of parasitized cells in young chicks injected intramuscularly with 0.5 cc male duck plasma daily from Nov. 18–Nov. 26, and inoculated intravenously with* 2.5×10^8 *chick erythrocytes infected with* Plasmodium lophurae *on Nov. 30.*

Series of chicks	Kind of data	Time after injection of parasitized duck cells							
		1 hr	1 da	2 da	3 da	4 da	5 da	6 da	7 da
Saline recipients (controls)	Mean	0.98	2.04	3.9	12.4	32.9	59.1	43.5	17.1
	S.D. (±)	0.21	0.39	0.6	4.1	7.6	10.2	4.1	4.4
Duck plasma recipients (tests)	Mean	0.98	1.86	3.6	9.5	25.6	31.3	14.5	6.3
	S.D. (±)	0.38	0.45	1.8	5.8	11.6	13.8	12.3	9.0

Differences between means highly significant on days 5 and 6.

parently, near-perfect harmony with normal plasma, pre-existing antibodies and cellular elements.

It is well known that the primary parasitemia rises to heights that produce severe effects in ducklings and adult ducks with *Plasmodium lophurae* (Wolfson, 1941). The speaker (1951) has found that goslings are, if anything, even more susceptible. Judging by parasitemia, this protozoan parasite tolerates ducks better than chicks, and chicks much better than adult chickens. It is said that it hardly tolerates the canary at all, or at best very poorly. Why are ducks better hosts for *P. lophurae* than chickens, or canaries? If the foregoing hypothesis concerning relapse is reasonably sound, the major part of the explanation may consist of the fact that duck seromucoid is a close functional analog of an antigen in the protoplasm of *P. lophurae*. As such it competes for antibody slightly more successfully, and for this reason spares the parasite more consistently, than homologous components in the plasma of adult chickens and canaries.

It is too much to expect that all details of the hypothesis are valid, or that there are not other considerations involved, such as agglutinins, precipitins and hemolysins, but there is reason to believe that in a general way it may explain the ability of malarial parasites to tolerate the hostile conditions they encounter inside their hosts, and to make those impressive curtain-calls which are called relapses, recurrences, or recrudescences. Incidentally, if one were so inclined, he could construct

a similar and more-or-less plausible hypothesis about the fundamental nature of cancer, one in which the survival and reproduction of the seemingly unruly neoplastic cells, originally produced through either somatic mutation or randomly, would be attributed in part to the sparing action of blood seromucoid known for certain to be present in abnormally excessive amounts in cancer patients. Tyzzer (1916) has compared neoplasms with parasites living at the expense of the host. How much of the blood seromucoid of cancer patients is contributed by the parasitic neoplasm, how much is in the predisposition of the host, and how much is from interaction of host and neoplasm are not known. The persistency of tuberculosis also could conceivably be due to the sparing effect of the excess of seromucoid in the blood of the patient on the responsible microorganism.

It seems fairly safe to conclude at this point that parasites tolerate their hosts, to a considerable degree at least, by virtue of certain of the polysaccharide compounds they fabricate, or by masquerading behind the mucoid materials of their hosts that are functionally, and perhaps chemically, similar to their own. One could envision also a battle of the protein-sugars, say, between the cuticle and hypodermal secretions of an ascarid and the intestinal mucus of the host; or a free-for-all melee among a half-dozen young taenias lustily bathing each others cuticles with their own auto-specific mucoid secretions until all are eliminated but one survivor, who becomes King Solium so long as he can tolerate the intestinal mucus that pervades his realm! It should be said, parenthetically, that we know very little about the occurrence of polysaccharide-containing compounds in the limiting membranes or inside the bodies of the parasitic protozoa. Certain free-living protozoa, however, are capable of secreting tectin, a mucoid material sometimes utilized in envelopes or skeletons, and sometimes for adhering to surfaces (Hyman, 1940).

Let it not be taken for granted that other free-living animals lack the protective, buffering materials comparable to those whose role in parasites have been under discussion. On the contrary, Baer (1951) has pointed out that the cuticles of all free-living nematodes possess the same fundamental structure as that in parasitic species, and that many of them live saprozoically in decomposing organic matter, which certainly contains enzymes. Bushnell and Erwin (1949) comment that while the tissues of *Ascaridia* from chickens, a worm which in life is constantly exposed to a tryptic enzyme, are about twenty times as active in inhibiting the action of the enzyme trypsin as are those of the earthworm *Lumbricus,* the "antitrypsin" (not identified) in the latter animal may serve the dual purpose of protecting it from its own tissue enzymes as well as from those of proteolytic bacteria in the soil flora. Evidently, then, the buffering materials of parasites represent preadaptations which their soil-dwelling, saprozoic or coprozoic antecedents found indispensable in their way of life. It has actually been suggested that parasites have come up through the ranks of saprozoic or coprozoic organisms.

The role mucoproteins and other polysaccharide compounds play in making parasites tolerant of some of the hostile factors in their special environments within hosts is exceedingly interesting and challenging academically, but I wish to drop this hint to the more practical minded individuals who may have found this dissertation somewhat dull—in your casting about for new approaches to the development of more effective parasiticidal drugs, consider the protein-sugars and their compounds elaborated by parasites. To what extent are these buffering materials,

which in polymerized form compose cuticles, capable of detoxifying drugs which are intrinsically toxic to the protoplasm of the parasite? Because cuticles appear glabrous and relatively stable does not mean that they are chemically inert. If that bothers you, it will help to compare them with ionic-exchange resins with whose activity you may be more familiar. In this connection let me remind you that Sternburg and Kearns (1952) have recently shown that DDT applied topically on the cuticle of a grasshopper is degraded, in the cuticle, to DDE as it is absorbed, so that DDT (dichloro-diphenyl-trichloroethane), the toxic compound, is not found in the tissues beyond the "cuticle-hypoderm," only its non-toxic metabolite, DDE (dichloro-diphenyl-dichloroethylene), being transported. It is not implied that protein-sugars are known for certain to function in the alteration of this toxic molecule, but the possibility remains, for A. G. Richards (1951) states in his book on the integument of arthropods that the older concept of arthropod cuticle as a chitinous matrix impregnated with other materials is gradually being supplanted by the view that the cuticle is a mucopolysaccharide, a combination of chitin and protein.

Going still further, there is the possibility of discovering substances nontoxic to the host which could successfully compete for the active groups in molecules of the protective mucoproteins of the parasite, and thus render them unable to play their accustomed role of neutralizing enzymes and antibodies, and detoxifying various otherwise injurious compounds. Flukes, tapeworms, nematodes or acanthocephalans with chemically inactivated cuticle, and elemental mucoid secretions of the cuticle-forming hypoderm seeping through the cuticle, would not tolerate for long the proteolytic enzymes, antibodies and other hostile agents of the host, to say nothing of host tissues reactions and the activities of phagocytic cells.

Another important practical possibility is introducing antigenic mucopolysaccharides or protein-sugars into the host for the purpose of eliciting either prophylactic or curative antibodies against particular parasites. It is to be recalled that the slimy coating of the pneumococcus, which is its defense against the attacks of its host, contains the very polysaccharide against which the host may eventually develop a solid immunity. Do, by analogy, the very polysaccharide compounds which are so indispensable for a time in shielding the animal parasite from the host eventually react to the detriment of the parasite by stimulating the production of antibodies? Certain past failures to protect animals by injecting them with suspensions or extracts of parasite tissues should not deter parasitologists from more persistent efforts in the same direction. It has already been mentioned that chicks, subjected to a series of intramuscular injections with duck plasma from ducks that never had malaria, acquired a partial active immunity to *Plasmodium lophurae*. We intend to find out some day which, if any, of the capsular materials from various sorts of bacteria, when injected into chickens or ducks, are capable of inducing protective antibodies against avian plasmodiums, and, if we are successful in this, other avian parasites.

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FIVE NEW SPECIES OF *ACANTHOBOTHRIUM* (CESTODA: TETRAPHYLLIDEA) FROM SOUTHERN CALIFORNIA RAYS

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A study has been made of the tetraphyllidean cestodes inhabiting the spiral valve of *Rhinobatos productus* (Ayres) and *Holorhinus californicus* (Gill). The present paper includes only the species of *Acanthobothrium* van Beneden, 1850, occurring in these hosts in Southern California waters. *Rhinobatos productus* was found to harbor two species, and *Holorhinus californicus,* three species of *Acanthobothrium.* All of these cestodes seem to represent species previously unknown to science.

Acanthobothrium microcephalum n. sp. (Plate I, Figs. 1, 2, 3)

Medium sized, fragile, acraspedote, apolytic worms. Single specimen measuring 61 mm. in length (approximately 240 proglottids) and a maximum width of 0.47 mm. Immature proglottids quadrate, 0.41 mm. wide. Mature proglottids slightly longer than broad, 0.43 to 0.47 mm. wide by 0.59 to 0.63 mm. long. Holdfast considerably narrower than neck, 0.44 mm. wide at level of hooks. Bothridia 0.22 to 0.23 mm. wide by 0.36 to 0.41 mm. long. Posterior half of each bothridium essentially free, attached to holdfast stalk by loose velum-like fold. Hooks inserted above anterior margins of bothridia in muscular pad whose apex bears small accessory sucker. Hooks small, prongs of equal length. Each hook with tubercle at base of inner prong. Total length of hooks 100 μ; handle to bifurcation 36 μ; prongs 64 μ long. Neck 0.81 mm. wide at its midpoint, 2.20 mm. long. Testes spherical to sub-spherical in shape, overlapping slightly; testes 90 to 100 in number, 23 to 38 μ in diameter. Vas deferens a large tube forming a mass of coils in anterior half of proglottid in mature segments. Cirrus pouch small, rounded, extending inward through one-fourth to one-third of proglottid width; pouch 0.08 to 0.10 mm. wide by 0.13 to 0.17 mm. long. Cirrus armed with fine, hair-like spines. Genital aperture marginal, in posterior third of proglottid, irregularly alternating. Vagina large, crossing vas deferens at proximal end of cirrus pouch. Ovary with large, club-shaped lobes extending forward to level of cirrus pouch; lobes connected posteriorly by narrow isthmus. Vitellaria consisting of narrow band of laterally located follicles on each side extending through length of proglottid.

Host: *Holorhinus californicus* (Gill).
Location: Spiral valve.
Locality: Long Beach Harbor, California.
Type specimen: U.S.N.M., Helm. Coll. No. 47852.

This species most closely resembles *Acanthobothrium uncinatum* Rudolphi, 1819, as described by Southwell (1925), in the total hook length and body length. However, there are several points of difference: the inner prongs of the hooks of *A. uncinatum* are considerably longer than the outer prongs, while those of *A. microcephalum* are of equal length; the number of testes per proglottid in *A. microcephalum* is about twice that of *A. uncinatum;* mature proglottids of *A. uncinatum* are about twice as long as broad, while those of the new species are only slightly longer than broad.

Acanthobothrium holorhini n. sp. (Plate I, Figs. 4, 5, 6)

Small, acraspedote, apolytic worms up to 24 mm. long with maximum width of 0.43 mm. and consisting of 63 to 80 proglottids. Immature proglottids slightly wider than long to quadrate,

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0.17 to 0.37 mm. wide by 0.10 to 0.16 mm. long; mature proglottids twice as long as broad, 0.35 to 0.43 mm. wide by 0.82 to 1.42 mm. long. Holdfast 0.30 to 0.36 mm. wide by 0.37 to 0.46 mm. long. Bothridia 0.16 to 0.22 mm. wide by 0.42 to 0.53 mm. long. Posterior half of each bothridium essentially free, attached to holdfast stalk by loose velum-like fold. Hooks inserted above anterior margins of bothridia in muscular pad whose apex bears an inconspicuous accessory sucker. Outer prong of each hook slightly longer than inner prong; inner prong bears prominent tubercle at base near point of bifurcation. Hooks bear several minute projections on surface near point of bifurcation. Total length of hooks 191 to 218 μ; handle to bifurcation 64 to 73 μ; outer prong 129 to 140 μ; inner, 102 to 127 μ. Neck 2.30 to 5.40 mm. long. Testes 60 to 77 per proglottid, 62 to 77μ in diameter, in one field. Vas deferens forms narrow, median mass of coils in anterior half of proglottid. Genital aperture at middle of proglottid margin; irregularly alternating. Vagina relatively large, turning posteriorly at medial end of cirrus pouch. Cirrus pouch small, extending inward through one-third width of proglottid. Cirrus armed with fine, hair-like spines. Ovary consists of narrow lobes connected posteriorly by narrow isthmus; each lobe extends forward to level of cirrus pouch. Vitellaria consists of narrow band of follicles on each side, extending from behind ovary throughout length of proglottid.

Host: *Holorhinus californicus* (Gill).
Location: Spiral valve.
Locality: Long Beach Harbor, California.
Holotype: U.S.N.M., Helm. Coll. No. 47853.
Paratype: U.S.N.M., Helm. Coll. No. 47854.
Additional specimen deposited in Helminthological Collection, Dept. of Zoology, University of California, Los Angeles.

The total hook length and hook dimensions of this species place it close to *Acanthobothrium coronatum* Rudolphi, 1819. However, comparisons of the present species with dimensions of *A. coronatum,* as given by Southwell (1925) and Baer (1948), indicate some differences between the two. The body length of *A. coronatum* is greater and the strobila much more robust and heavily muscled, a characteristic diagnostic feature of this species. In addition, the testes in *A. coronatum* number from 80 to 100, while those of *A. holorhini* number from 60 to 77. Without morphological evidence it might be suspected that the two species are not identical since *A. coronatum* is one of the three species characterized in Baer's key (1948) as being confined to sharks in contrast with those found in rays.

Acanthobothrium unilateralis n. sp. (Plate I, Figs. 7, 8, 9)

Small, acraspedote, apolytic worms measuring up to 19.50 mm. in length and 0.54 mm. in maximum width. Only the last two or three (of approximately 50) proglottids mature. Mature proglottids approximately twice as long as broad, 0.49 to 0.54 mm. wide by 1.03 to 1.12 mm. long. Bothridia relatively long with anterior loculi making up about half the length. Posterior half of each bothridium essentially free, attached to holdfast stalk by loose velum-like fold. Bothridia 0.68 to 0.75 mm. in length. Each pair of hooks inserted at tip of bothridium in base of triangular, muscular pad whose apex bears a small accessory sucker. Hooks small relative to holdfast size; prongs approximately equal in length; inner prong bearing a tubercle near its base; handle bearing several minute projections on outer surface. Total hook length 173 to 182 μ; handle 58 to 64 μ; prongs 118 μ. Neck 2.10 to 5.00 mm. in length, covered with minute hair-like spines. Testes 40 to 50 in number, sub-spherical, closely packed and filling proglottid between vitellaria; diameter 82 to 91 μ. Vas deferens a large tube with several coils in midfield. Cirrus pouch relatively small, extends inward through approximately one-third width of proglottid, 0.14 mm. wide by 0.15 to 0.16 mm. long. Cirrus armed with minute spines. Genital atrium in middle of proglottid margin; openings unilateral. Vagina with weakly developed sphincter at its opening, enlarges immediately before turning posteriorly at medial end of cirrus pouch. Ovary with two narrow lobes, connected by narrow isthmus; poral lobe extends forward to cirrus pouch; aporal lobe extends forward through half of length of proglottid. Vitellaria consists of narrow band of follicles on each side, extending throughout entire length of proglottid.

Host: *Holorhinus californicus* (Gill).
Location: Spiral valve.
Locality: Long Beach Harbor, California.
Type specimen: U.S.N.M., Helm. Coll. No. 47855.

This species differs from all other members of the genus in the unilateral nature of the gonopore openings. In most other respects it resembles *Acanthobothrium crassicole* Wedl, 1855, as described by Southwell (1925). The description of *A. crassicole* by Baer (1948) shows little agreement with that of Southwell. However, neither Baer's nor Southwell's description mentions a spiny neck covering, which feature, in addition to the unilateral gonopores, serves to differentiate *A. unilateralis* from *A. crassicole*.

Acanthobothrium robustum n. sp. (Plate II, Figs. 10, 11, 12)

Medium sized, acraspedote, apolytic worms up to 59.50 mm. in length; maximum width 0.63 mm.; proglottids 106 to 113 in number. Immature proglottids quadrate to slightly longer than broad, 0.25 to 0.31 mm. wide by 0.25 to 0.33 mm. long; mature proglottids slightly longer to twice as long as broad, 0.37 to 0.63 mm. wide by 0.75 to 1.17 mm. long. Holdfast robust, 0.41 to 0.56 mm. wide. Bothridia oval, muscular, with terminal tips free, remainder attached to holdfast by loose, velum-like fold. Each bothridium bears a pair of large, muscular, forwardly-directed accessory suckers at apex. Bothridia 0.19 to 0.29 mm. wide by 0.31 to 0.44 mm. long. Accessory suckers 94 to 112 μ in diameter. Hooks small, stout, with irregular edges; inner prong much larger than outer prong; accessory spur, resembling hook prong, projects from hook at base of outer prong. Total hook length 135 to 150 μ; handle to bifurcation 45 μ; inner prong 78 to 90 μ; outer prong 52 to 67 μ. Testes small, 99 to 101 in number, 31 to 38 μ in diameter. Vas deferens forms a mass of coils in mid-field between lobes of ovary. Cirrus pouch small, oval, length little more than one-fourth width of proglottid, 0.11 to 0.13 mm. wide by 0.16 to 0.19 mm. long. Genital atrium slightly posterior to middle of proglottid margin; openings irregularly alternating. Ovary in two widely separated lobes anteriorly, connected posteriorly by narrow isthmus; ovary 0.38 to 0.44 mm. wide by 0.28 to 0.31 mm. long. Vitellaria consists of narrow band of follicles on each side, extending throughout entire length of proglottid.

Host: *Rhinobatos productus* (Ayres).

Location: Spiral valve.

Locality: Long Beach Harbor, California.

Holotype: U.S.N.M., Helm. Coll. No. 47856.

Paratype: U.S.N.M., Helm. Coll. No. 47857.

Additional specimen deposited in Helminthological Collection, Dept. of Zoology, University of California, Los Angeles.

The two large accessory suckers on each bothridium and the distinctive hook shape readily distinguish this species from the other species of the genus.

Acanthobothrium rhinobati n. sp. (Plate II, Figs. 13, 14, 15)

Small, acraspedote, apolytic worms up to 32 mm. in length with maximum width of 0.54 mm. Strobila consists of approximately 50 proglottids. Immature proglottids slightly broader than long, 0.30 to 0.32 mm. wide by 0.21 to 0.23 mm. long. Mature proglottids approximately twice as long as broad, 0.50 to 0.54 mm. wide by 1.09 to 1.31 mm. long. Holdfast relatively large, with bothridia muscular and loculi well defined; holdfast 0.40 to 0.71 mm. wide by 0.44 to 0.63 mm. long. Bothridia 0.23 to 0.30 mm. wide by 0.41 to 0.60 mm. long. Posterior half of each bothridium essentially free, attached to holdfast stalk by loose velum-like fold. Hooks slender and attached to base of muscular pad whose apex bears small accessory sucker; tubercle present at base of inner prong of hook; inner prong slightly longer than outer prong. Total length of hooks 113 to 127 μ; outer prong 73 to 75 μ; inner prong 78 tto 85 μ; handle 29 to 36 μ. Neck slender, 3.60 to 5.10 mm. long, covered with minute hair-like spines. Testes 51 to 62 in number, spherical, approximately 46 μ in diameter in mature proglottids, filling proglottid from ovary forward in single field. Cirrus pouch large, extends halfway across proglottid, 0.15 to 0.19 mm. wide by 0.21 to 0.24 mm. long. Cirrus heavily armed with fine spines. Genital aperture midway along lateral margin of proglottid; openings irregularly alternating. Vagina large, bearing a prominent sphincter at its opening into genital atrium, passing posteriorly around medial end of cirrus pouch. Ovary consists of narrow lobes connected posteriorly by narrow isthmus; lobes extend forward to level of cirrus pouch. Vitellaria consists of a narrow band of compact follicles on each side, extending forward throughout most of length of proglottid.

Host: *Rhinobatos productus* (Ayres).

Location: Spiral valve.

Locality: Santa Monica Harbor, Ocean Park Pier, California.
Holotype: U.S.N.M., Helm. Coll. No. 47858.
Paratype: U.S.N.M., Helm. Coll. No. 47859.
Additional specimen deposited in Helminthological Collection, Dept. of Zoology, University of California, Los Angeles.

This species was present in considerable numbers in the spiral valve of a single host taken at Ocean Park Pier. It closely resembles *Acanthobothrium benedenii* Loennberg, 1889 (*benedeni* of Baer (1948)), the only major differences being the larger over-all size and greater number of proglottids in *A. rhinobati.* In addition, *A. rhinobati* has a larger number of testes and larger hooks, in terms of average measurement, than *A. benedenii.*

A conspicuous feature, which all of the species have in common and which is probably characteristic of the genus *Acanthobothrium,* is the nature of the neck musculature. Although the bodies of the worms have a poorly developed musculature, the necks in all cases possess a well developed musculature which is composed of eight distinct bundles extending up into the holdfast. Paired bundles apparently are inserted in the bothridial apparatus; one bundle is attached to the bothridium proper, and one to the hooks and the muscular pad in which the hooks lie.

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Plate I

Acanthobothrium microcephalum n. sp. Figs. 1, holdfast; 2, Mature proglottid; 3, Hook.
Acanthobothrium holorhini n. sp. Figs. 4, Holdfast; 5, mature proglottid; 6, Hook pair.
Acanthobothrium unilateralis n. sp. Figs. 7, Holdfast; 8, Mature proglottid; 9, Hook pair.

PLATE I

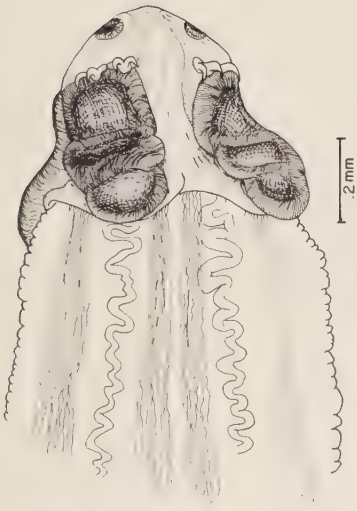

FIG. 1

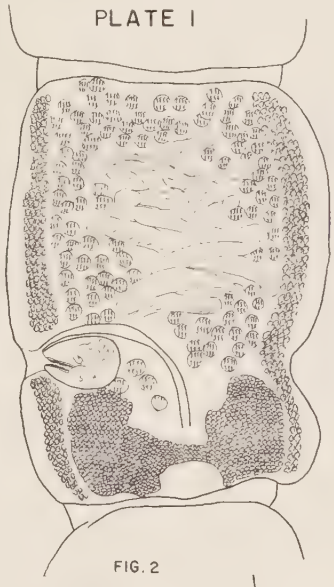

FIG. 2

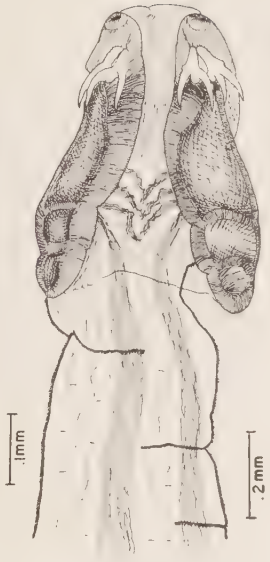

FIG. 7

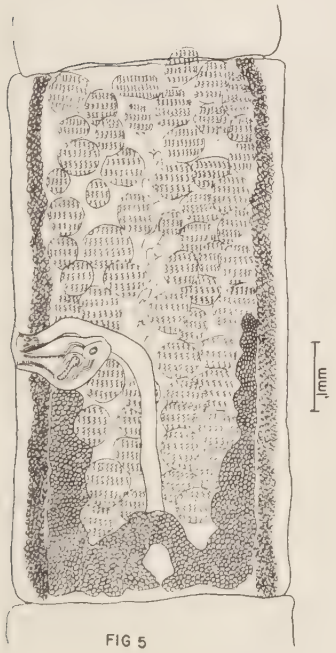

FIG 5

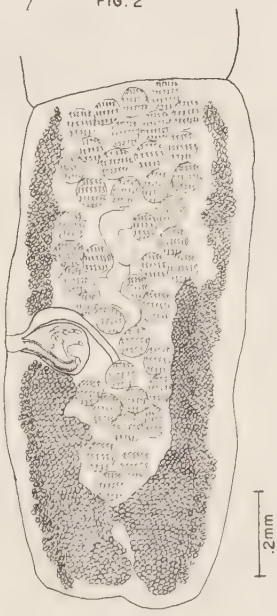

FIG. 8

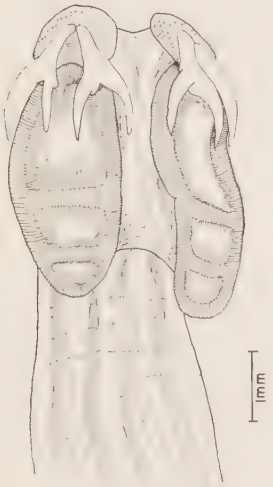

FIG. 4

FIG. 3

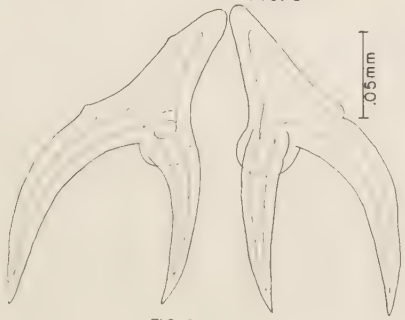

FIG. 6

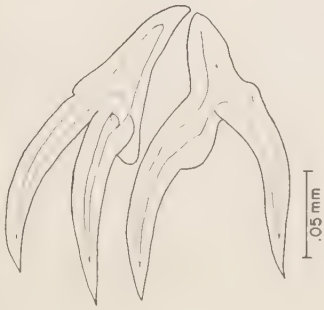

FIG. 9

PLATE II

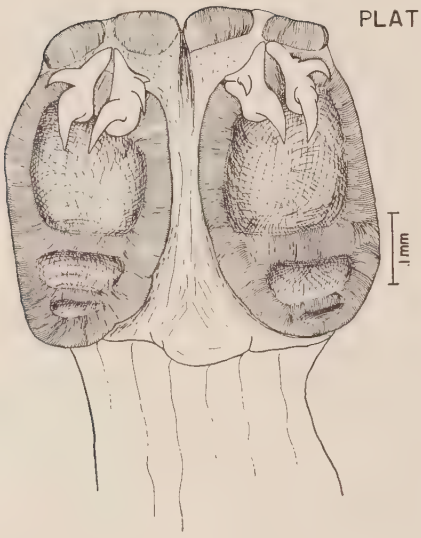

FIG. 10

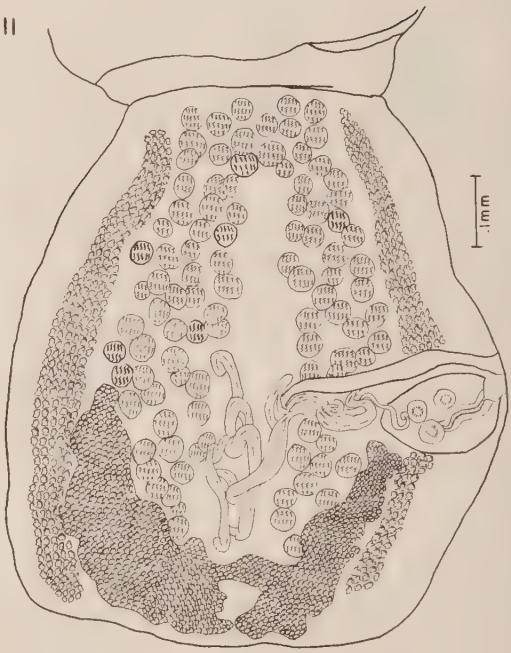

FIG. 11

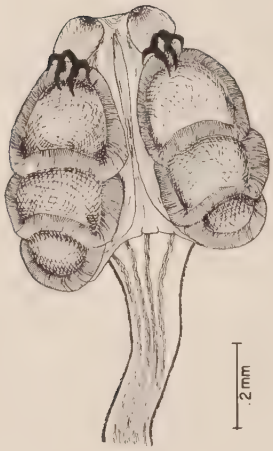

FIG. 13

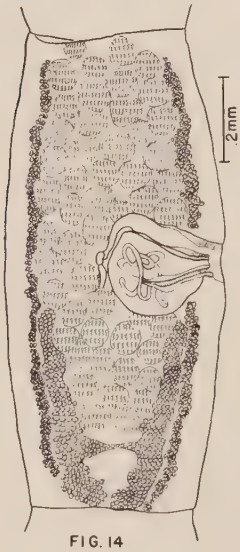

FIG. 14

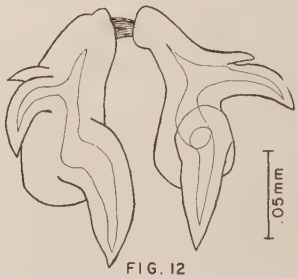

FIG. 12

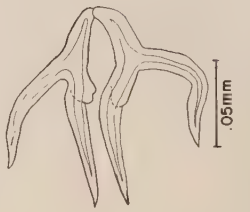

FIG. 15

PLATE II

Acanthobothrium robustum n. sp. FIGS. 10, Holdfast; 11, Mature proglottid; 12, Hook pair.
Acanthobothrium rhinobati n. sp. FIGS. 13, Holdfast; 14, Mature proglottid; 15, Hook pair.

HELMINTHS OF NORTHWESTERN MAMMALS. PART V. OBSERVATIONS ON CESTODES OF SHREWS WITH THE DESCRIPTIONS OF NEW SPECIES OF *LIGA* WEINLAND, 1857, AND *HYMENOLEPIS* WEINLAND, 1858

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The cestodes recorded in this study represent a partial review of a large number of individuals recovered from thirteen specimens of three species of shrews, Palmer Marsh Shrew, *Sorex bendirii palmeri* Merriam (7 specimens); Cascade Dusky Shrew, *S. obscurus permiliensis* Jackson (2 specimens); and Vagrant Shrew, *S. v. vagrans* Baird (4 specimens). Neither of the former two shrews have been previously reported in the literature as harboring animal parasites, while only recently Locker and Rausch (1952) published a report on the helminths of the Vagrant Shrew. One of the specimens of *S. bendirii palmeri* harbored along with its probable other intestinal fauna the following kinds of helminths: 7 species of cestodes, 5 species of trematodes, 2 species of nematodes, and an acanthocephalan. A conservative estimate of the total number of these fifteen species of helminths would probably exceed 1000. Even so the host weighed about 14 per cent more than the heaviest North American long-tailed shrew recorded by Jackson (1928) and from 43 to 76 per cent more than any of the other specimens of this species collected during the study. Gross examination of its viscera failed to reveal any evident pathology and it seems apparent that the host was able to maintain adequate nutrition even though burdened by its helminth dependents.

Whole mounts were prepared from specimens stained in either Semichon's aceto-carmin or Kornhauser's hematin and where necessary, serial sections were prepared and stained with hematoxylin and eosin. Unless otherwise noted, all measurements are given in millimeters. Drawings were prepared with the aid of a camera lucida.

The writer wishes to thank Mr. Clyde M. Senger, Department of Biological Sciences, Purdue University, for his help in collecting and preparing some of the material. Also thanks are due Dr. Ralph W. Macy for the use of the Reed College Biology Laboratories, and for the use of his personal reprint collection. Dr. Robert Rausch, Arctic Health Research Center, Anchorage, Alaska, kindly identified some of the shrews, while the remainder were identified by the staff of the U. S. National Museum.

Protogynella blarinae Jones, 1943

This minute cestode, previously reported from the northwestern shrew, *Sorex v. vagrans*, by Locker and Rausch (1952) and from the eastern shrews, *S. c. cinereus* Kerr and *Blarina brevicauda* Say, by Rausch and Kuns (1950), is recorded here from two specimens of *S. bendirii palmeri*, one from Larch Mountain and one from Mt. Hood, Oregon.

Hymenolepis intricatus Locker and Rausch, 1952

This cestode was recovered from two specimens of *S. bendirii palmeri*, one taken at Larch Mountain and the other at Mt. Hood, Oregon. This species was recorded previously from *S. v. vagrans* by Locker and Rausch (1952). The present material agrees with the original descrip-

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tion in all respects except the length of hooks. The hooks reach a length of 0.026 while the original description gives an upper limit of 0.021. This was one of the least frequent cestodes observed.

Hymenolepis kenki Locker and Rausch, 1952

Hymenolepis kenki, originally described from *S. v. vagrans,* was found in two *S. bendirii palmeri* and one *S. obscurus permiliensis* from Larch Mountain, Oregon.

Hymenolepis macyi Locker and Rausch, 1952

The cestode was observed in two *S. bendirii palmeri* from Larch Mountain and one from Mt. Hood, Oregon. It was previously reported from *S. v. vagrans.*

Hymenolepis longi, Oswald, 1951

Originally described from the smoky shrew (*S. fumeus* Miller), of the eastern United States, *Hymenolepis longi* was one of the most frequently observed cestodes. It was recovered from five of the seven *S. bendirii palmeri* taken at the Larch Mountain, Mt. Hood, and Eagle Creek localities. A careful examination of these specimens revealed differences from the original description in only one respect. In all the specimens studied (15), the rostellum was found to be armed with ten instead of eight hooks. A study of additional eastern specimens, as well as more western specimens from other hosts and localities, will probably be necessary before it can be determined if two separate species are involved.

Hymenolepis sengeri n. sp. (Plate I, Figs. 1–4)

Diagnosis: Strobila up to 20 long and 0.37 broad. Margins of strobila serrate only in gravid region, where proglottids are bell-shaped. Scolex moderately developed, about 0.15–0.22 broad; suckers oval, 0.14–0.19 long by 0.09–0.11 broad. Rostellum short and broad, armed with hooks and separated from base by definite constriction, 0.13–0.18 long by 0.09–0.01 broad. Rostellum armed with 10 hooks, 0.051–0.056 long. Genital pores unilateral, dextral; situated in anterior half of proglottid. Cirrus pouch elongate, reaching about one-third across proglottid, 0.08–0.12 long by 0.02 broad. Cirrus spinose. Weakly developed external seminal vesicle present. Testes in middle of proglottid arranged in a triangle; middle and left testes in line and anterior to right testis. Vagina ventral to cirrus pouch. Small seminal receptacle present. Ovary posterior and somewhat to left of middle testis, about same size or slightly larger than a testis. Vitelline gland posterior to left testis, smaller than a testis. Uterus sacculate, filling central region of gravid proglottid. Eggs round or slightly oval, 31–40 μ in diameter.

Host: Sorex bendirii palmeri Merriam (Palmer Marsh Shrew).

Locality: Larch Mountain and Mt. Hood, Oregon.

Habitat: Small intestine.

Type: Slides Nos. 47864 and 47865 bearing type and paratype specimens have been deposited in the helminthological collection, U. S. National Museum.

Incidence: Collected from one of three shrews from Mt. Hood and from three of five from Larch Mountain.

Hymenolepis sengeri n. sp. can be differentiated from the other closely related soricid species of cestodes having 10 hooks by the size and shape of the hooks, size and extent of cirrus pouch, distribution of testes, and size of eggs. *H. singularis* Cholodkowsky, 1912, has larger hooks (0.061–0.062), smaller overall size (2–5), testes in a straight line, and oval eggs (0.053 by 0.024). *H. scutigera* (Dujardin, 1845) has smaller hooks (0.033–0.040), testes in straight line, a much smaller cirrus pouch (0.040 by 0.010), and a crescent-shaped uterus. *H. parva* Rausch and Kuns, 1950, has smaller hooks (0.034–0.040), testes in straight line, and smaller eggs (0.020–0.025). *H. blarinae* Rausch and Kuns, 1950, has smaller hooks (0.033) of different shape, and a much greater overall size (90 by 1). *H. lineola* Oswald, 1951, has much smaller hooks (0.0308–0.0324) and a smaller cirrus pouch (0.055–0.069 by 0.011–0.013).

This species is named in honor of Mr. Clyde M. Senger, who helped collect and prepare this material for study.

Hymenolepis pauciproglottis n. sp. (Plate II, Figs. 5–8)

Diagnosis: Strobila from 1.15–1.80 long by 0.34–0.48 broad, composed of up to 8 proglottids, one or two of which may be gravid. Margins of strobila serrate. Scolex well developed, about 0.18 broad. Suckers oval, 0.11 long by 0.08 broad. Rostellum short and heavily built, 0.10 long by 0.07 broad; armed with 12 hooks 0.012–0.014 long. Genital pores unilateral, situated in anterior third of segment. Cirrus pouch very large, sigmoid-shaped, extending well past middle of proglottid, from 0.13–0.15 long by 0.02 broad. Small internal and large external seminal vesicles present; latter bends back over cirrus pouch. Cirrus armed at its base with heavy spines. Testes situated in a straight line along posterior border of proglottid. Ovary bilobed, situated in middle of proglottid near anterior margin. Vagina ventral to cirrus pouch. Small seminal receptacle present. Vitelline gland posterior to isthmus of ovary and anterior to middle testis. Uterus sacculate, filling proglottid in gravid segments. Embryo 20–24 μ in diameter.

Host: Sorex v. vagrans Baird (Vagrant Shrew).

Locality: Todd Lake, Bend, Oregon.

Habitat: Small intestine.

Type: Slides Nos. 47862 and 47863, bearing type and paratype specimens, have been deposited in the helminthological collection of the U. S. National Museum.

Incidence: Collected from one of four shrews taken at Todd Lake, Bend, Oregon.

Hymenolepis pauciproglottis n. sp. can be readily differentiated from the only other soricid species of *Hymenolepis* having 12 hooks (*H. scalaris* Dujardin, 1845, *H. dodecantha* Baer, 1925, and *H. falculata* Rausch and Kuns, 1950) by differences in hook size and shape, cirrus pouch size and shape, testis arrangement, and its much smaller overall size.

Since the description of the first reported species of *Hymenolepis* from North American shrews (*H. anthocephalus* Van Gundy, 1935), eleven additional species have been recorded from shrews of this continent: four by Rausch and Kuns (1950), three by Oswald (1951), and four by Locker and Rausch (1952). In the present paper, two more species are added to the list, bringing the total to fourteen. In confirmation of the suggestion of Rausch and Kuns (1950), "that cestodes in North American shrew are strictly North American species," it can be pointed out that, since then, the examination of four additional species of shrews from new localities has failed to yield a single Eurasian species of *Hymenolepis*. Although the evidence pertaining to this matter can not be considered complete, it seems highly suggestive. With this unique feature of the hymenolepidid fauna of North American shrews in mind, it does not seem superfluous to include a key for their identification. Measurements are given in millimeters.

Key to Species of Hymenolepis In North American Shrews

1. Scolex armed 3
   Scolex unarmed 2
2. Strobila from 40–100 long; testes arranged in triangle. *H. anthocephalus* Van Gundy, 1935
   Strobila up to 2 long; testes in straight line; cirrus spinose; ovary tripartite; cirrus pouch extending almost across entire segment ..... *H. macyi* Locker and Rausch, 1952
   Strobila 1–2 long; testes in straight line; cirrus spinose; ovary subspherical; cirrus pouch extending only to midline ..... *H. kenki* Locker and Rausch, 1952
3. Rostellar hooks 22 in number, 0.027–0.030 long ..... *H. schilleri* Rausch and Kuns, 1950
   Rostellar hooks 12 in number 4
   Rostellar hooks 8–10 in number 5
4. Hooks 0.022–0.025 long; strobila 30–40 long ..... *H. falculata* Rausch and Kuns, 1950
   Hooks 0.012–0.014 long; strobila up to 1.8 long, consisting of only a few proglottids. *H. pauciproglottis* n. sp.
5. Rostellar hooks only 8 in number, 0.018–0.021 long ..... *H. serrula* Oswald, 1951
   Rostellar hooks 8 or 10 in number, 0.0206–0.0240 long ..... *H. longi* Oswald, 1951
   Rostellar hooks only 10 in number 6

6. Hooks 0.016–0.020 long; cirrus pouch extending beyond midline of segment.
H. sphenomorphus Locker and Rausch, 1952
Hooks 0.017–0.026 long; vagina with protruding terminal enlargement.
H. intricatus Locker and Rausch, 1952
Hooks 0.0308–0.0324 long; testes arranged in triangle; strobila 2–3 long.
H. lineola Oswald, 1951
Hooks 0.033 long; strobila up to 90 long ..... *H. blarinae* Rausch and Kuns, 1950
Hooks 0.034–0.040 long; strobila 3–5 long; testes in straight line.
H. parva Rausch and Kuns, 1950
Hooks 0.051–0.056 long; strobila up to 20 long; testes arranged in triangle.
H. sengeri n. sp.

With the exception of these fourteen species of *Hymenolepis,* only two other cestodes have been previously observed in North American shrews: *Protogynella blarinae* Jones, 1943, and *Diorchis reynoldsi* Jones, 1944. The former has been reported, subsequent to its description, by Rausch and Kuns (1950) from the type-host in southern Wisconsin and by Locker and Rausch (1952) from a Pacific coast shrew. The latter, however, is apparently a more local species and has not been reported outside of Virginia. *Liga soricis* n. sp. represents the fourth genus and second family of cestodes to be discovered in shrews on this continent.

Liga soricis n. sp. (Plate III, Figs. 9–11)

Diagnosis: Dilepidinae. Strobila up to 10 long with maximum breadth of 0.74 attained in gravid segments; there may be as many as 51 segments with serrate margins. Scolex well developed, from 0.47–0.55 broad. Suckers prominent, oval, from 0.28–0.41 long by 0.14–0.20 broad. Rostellum rather long, 0.34–0.42 long by 0.07–0.10 broad; rostellum armed with double row of 14–16 morphologically similar hooks from 0.027–0.033 long. Genital pores regularly alternating, located in anterior third of proglottid. Genital ducts pass dorsal to excretory vessels. Cirrus pouch weakly developed, extending not more than half its length beyond the excretory vessels, from 0.12–0.18 long by 0.02–0.03 broad. Cirrus long and spinose. Vas deferens about 5 μ in diameter, forms 2 or 3 loops inside cirrus pouch; vas deferens much heavier outside of cirrus pouch, about 11 μ in diameter, extending through a few loops to midline of proglottid. Testes 13–20 in number and all situated posterior to ovary and vitelline gland. Ovary bilobed, situated in the anterior half of proglottid. Vitelline gland triangular, located at the midline between ovary and testes. Vagina posterior to cirrus pouch, rather well developed near its opening into the genital atrium. Well developed seminal receptacle present. Uterus deeply lobed, filling gravid segments. Eggs approximately spherical, from 20–30 μ in diameter.

Host: Sorex bendirii palmeri Merriam. Also recorded from *S. obscurus permiliensis* Jackson (Larch Mountain, Oregon).

Locality: Eagle Creek, Mt. Hood, and Larch Mountain, Oregon.

Type: Slides Nos. 47866 and 47867, bearing type and paratype specimens, have been deposited in the helminthological collection of the U. S. National Museum.

Incidence: Collected from a single shrew from Eagle Creek, from two out of three from Mt. Hood, and from two out of five from Larch Mountain, Oregon.

Previous to this time, five other species of *Liga* Weinland, 1857, have been described. Without exception they are reported from birds. Although there are many examples of genera of helminths which parasitize both birds and mammals, it seems unusual that *Liga,* a genus only infrequently observed even in birds, should be found as a common parasite of certain shrews of this region. It is noted that *Liga brasiliensis* Parona, 1901, the only species from this hemisphere, bears a closer similarity to the new species than any other member of the group. However, when one considers the fact that the feeding habits of the hosts (woodpeckers and shrews) of *L. brasiliensis* and *L. soricis,* respectively, are quite similar (i.e. both feed predominantly on terrestrial insects), a fairly plausible explanation of this coincidence may be had. It does not appear entirely unlikely, that an avian species of parasite

could successfully invade a mammalion host, if the opportunity to do so were frequent and over a long enough period of time.

The following key can be used to separate *Liga soricis* n. sp. from the other members of the genus. Measurements are in millimeters.

Key To Species of Liga

1. Rostellar hooks 28 or more in number 2
   Rostellar hooks 26 or less in number 3
2. Hooks 48 in number, 0.007–0.008 long ..... *L. alterans* (Cohn, 1900)
   Hooks 28–30 in number, 0.050 long ..... *L. frigida* Meggitt, 1927
3. Hooks 22–26 in number, 0.030–0.035 long; 10–14 testes; cirrus unarmed; eggs 0.026 in diameter ..... *L. gallinulae* (Van Beneden, 1861)
   Hooks only 22 in number, 0.042 long; 18–20 testes; cirrus unarmed; eggs 0.037 in diameter ..... *L. facilis* (Meggitt, 1927)
   Hooks 20 in number, 0.039–0.050 long; 12–18 testes; cirrus spinose; eggs, 0.033–0.036 in diameter ..... *L. brasiliensis* Parona, 1901
   Hooks 14–16 in number, 0.027–0.033 long; 13–20 testes; cirrus spinose; eggs 0.020–0.030 in diameter ..... *L. soricis* n. sp.

SUMMARY

The results of the examinations of thirteen specimens of three species of Oregon shrews (*Sorex bendirii palmeri* Merriam, *S. obscurus permilcinsis* Jackson, and *S. v. vagrans* Baird) are reported. It is believed that this report records the first cestodes from the former two species. Five species of previously known soricid tapes are reported from these hosts and a new species of *Liga* Weinland, 1857, and two new species of *Hymenolepis* Weinland, 1858, are described. Additional information concerning the anatomy of *Hymenolepic longi* Oswald, 1951, and *H. intricatus* Locker and Rausch, 1952, is included. A key to the species of *Hymenolepis* in North American shrews and one for the separation of the members of the genus *Liga* are submitted.

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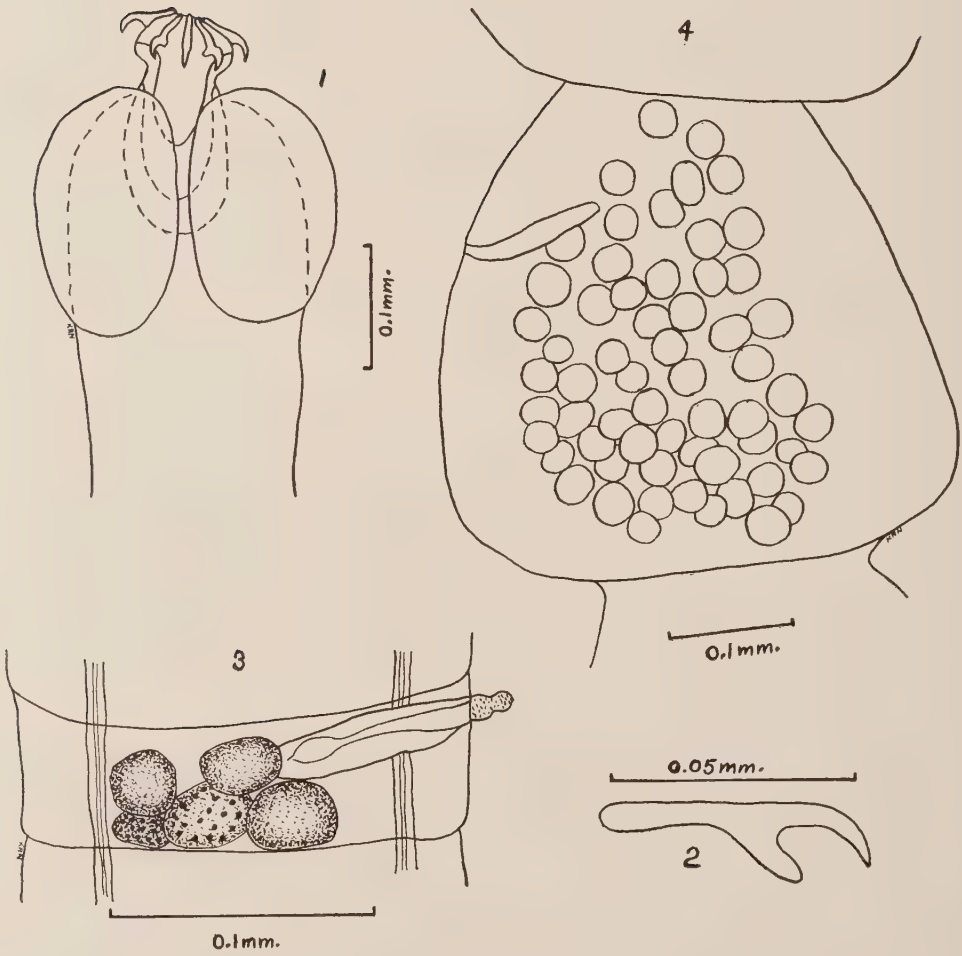


PLATE I

FIG. 1. *Hymenolepsis sengeri* n. sp., scolex.
FIG. 2. *H. sengeri* n. sp., hook.
FIG. 3. *H. sengeri* n. sp., mature proglottid.
FIG. 4. *H. sengeri* n. sp., gravid proglottid.

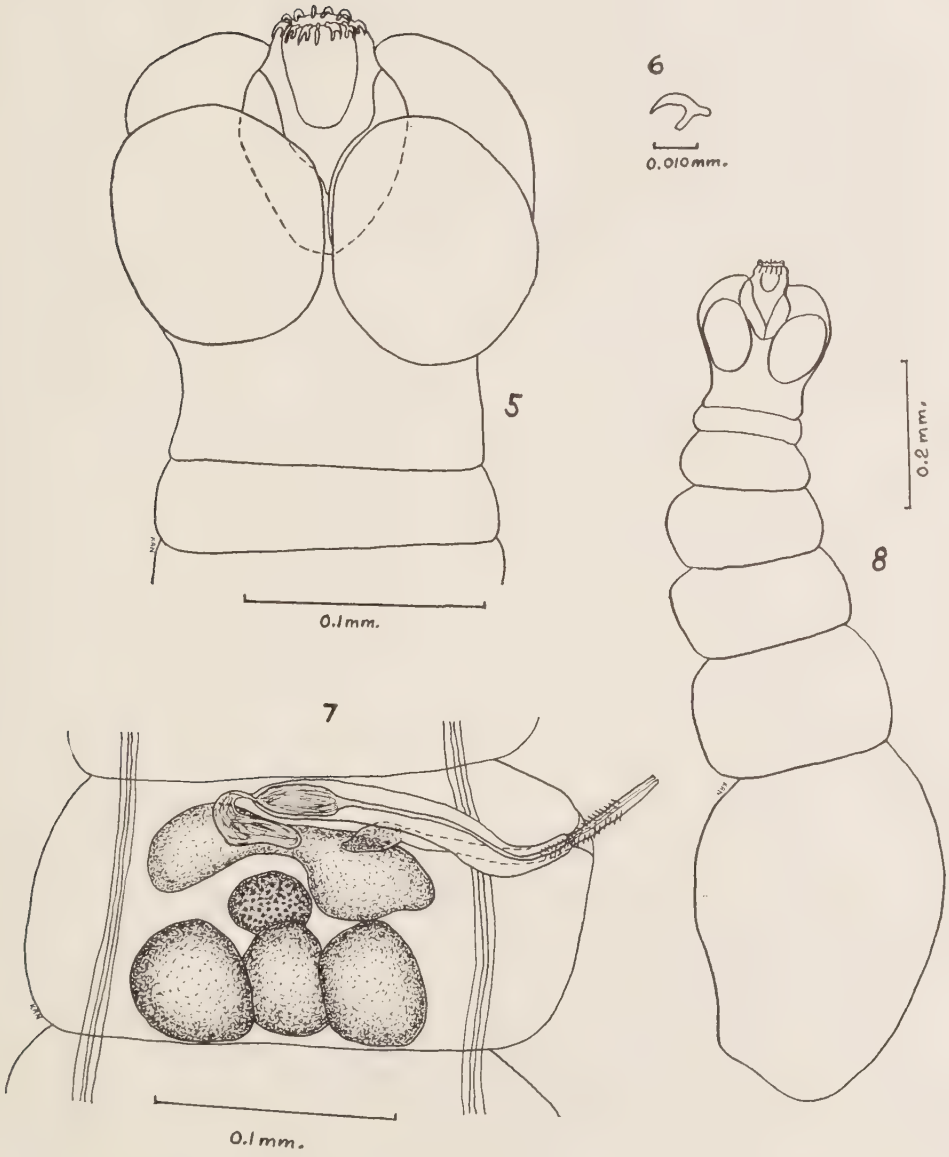
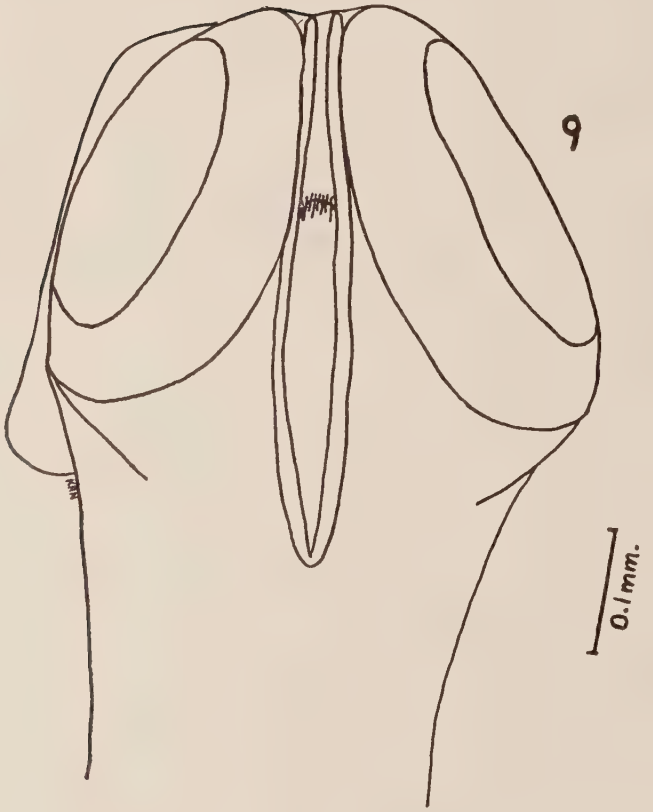
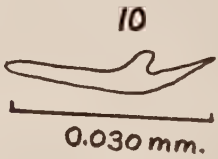


PLATE II

FIG. 5. *Hymenolepsis pauciproglottis* n. sp., scolex.
FIG. 6. *H. pauciproglottis* n. sp., hook.
FIG. 7. *H. pauciproglottis* n. sp., mature proglottid.
FIG. 8. *H. pauciproglottis* n. sp., view of entire strobila.





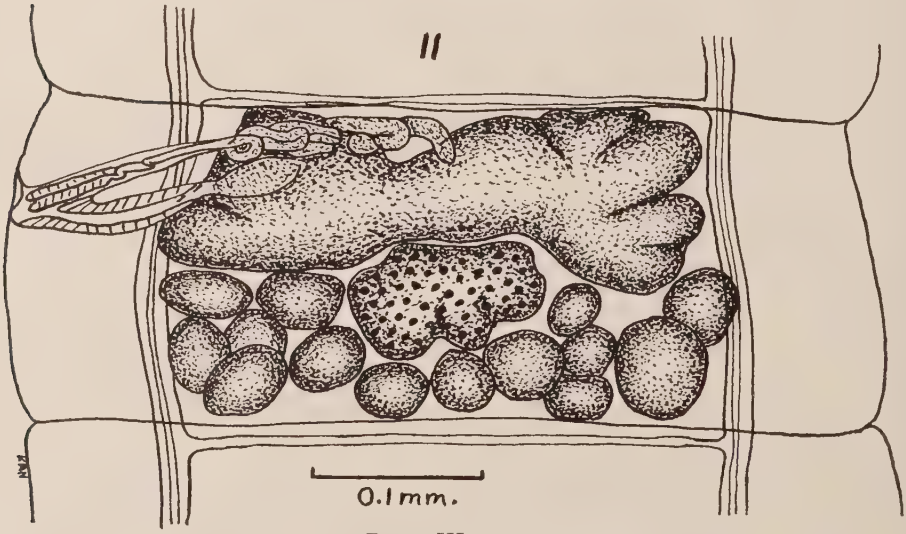


PLATE III

FIG. 9. *Liga soricis* n. sp., scolex.
FIG. 10. *L. soricis* n. sp., hook.
FIG. 11. *L. soricis* n. sp., mature proglottid.

NOTES ON THE RAT LICE *POLYPLAX SPINULOSA* (BURMEISTER) AND *HOPLOPLEURA OENOMYDIS* FERRIS

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Only two species of rat lice have been found commonly on domestic rats (*Rattus rattus* and *Rattus norvegicus*) in the United States since the Typhus Control Program of the State health departments and the Communicable Disease Center was inaugurated on July 1, 1945. The spiny rat louse, *Polyplax spinulosa* (Burmeister), has been known to occur on rats for over a century, while the tropical rat louse, *Hoplopleura oenomydis* Ferris, has been positively determined from this country only since 1937 and 1945 (Roudabush, 1939, and Pritchard, 1947). It is the purpose of this paper to figure both sexes, the eggs, and the three immature stages of these two species and to show the difference between them by means of tables. Notes on the distribution of the two species are included for the United States, Puerto Rico, and the Virgin Islands.

Methods of Study. It has been possible to observe pure cultures of both species of rat lice and to study the diagnostic characters of all stages by examining thousands of collections during the past 6 years. Laboratory colonies of both species were established, and the development of each species was followed on separate rats from egg to adult. It was possible to mount females of both species in various water-base mounts such as Berlese's fluid, polyvinyl alcohol, or lacto-phenol solutions, and to observe the differential characters of the egg stage of each species within the adult before the egg was laid, i.e., the rounded base of the egg and air pores across the center of the cap in *Polyplax,* or the projection at the base of the egg and the air pores around the periphery of the cap in *Hoplopleura.* Similarly, by working with large series of specimens, it was possible to find eggs and the three nymphal instars with the succeeding stage inside. By this technique it was possible to work out repeatedly the differential characteristics of each stage and the number of instars. Many of these interesting specimens have been cleared in caustic potash, run through a series of alcohols, cellosolve, and oil of cloves, and mounted as permanent balsam slides.

DIFFERENTIATION OF THE TWO SPECIES AND THEIR STAGES

The adults of *P. spinulosa* have been described and figured in detail by Ferris (1923) and Ferris and Stojanovich (1951) and some of the immature stages by Cummings (1915) and Ferris (1923). The adults of *H. oenomydis* have been described and figured in great detail by Ferris (1921), Ferris and Stojanovich (1951), and Pratt and Lane (1951), and the egg and one of the immature stages by Ferris (1932) and Ferris and Stojanovich (1951). It is worth noting here that the first instar nymph of *H. oenomydis* in figure 60 of Ferris and Stojanovich (1951) is actually a third stage nymph as reference to figure 39 of Ferris (1932) will reveal. The first stage nymph (fig. 8) in the present paper agrees with the statement by Ferris (1932, p. 126) that, "The first instar is represented . . . by an

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embryo in an egg and . . . the abdomen bears a single, very long seta on each side near the apex." Later instar nymphs lack this pair of long setae, as Ferris (1932) has shown. (See figures 9 and 10 of this paper.)

Similarly, neither of the first and second instar nymphs of *P. spinulosa* in figure 29 of Ferris and Stojanovich (1951) agrees entirely with those figured and described in Cummings (1915), in Ferris (1923), or in the present paper. The present study indicates that the first instar nymph has only a single large seta ventrally toward the tip of the abdomen, not two submedian longitudinal lines of setae ventrally as in figure 29 of Ferris and Stojanovich (1951). Neither the second nor the third stage nymph has more than two submedian longitudinal rows of setae dorsally or ventrally, whereas Ferris and Stojanovich (1951, fig. 29) show several transverse rows of setae dorsally and ventrally. It is possible that their drawing was made from a slide of a third instar nymph in which the setae of the developing adult showed through the integument of the nymph.

On gross inspection, adults of *P. spinulosa* appear somewhat more slender, and specimens in alcohol show more conspicuous indentations of the lateral margins of the abdomen than do those of *Hoplopleura*. All the immature stages of *H. oenomydis* have a characteristically heart-shaped abdomen but lack spiracles and submedian lines of setae on the abdomen. All the immature stages of *Polyplax* have a longer, more slender abdomen with spiracles and submedian lines of setae. Second and third stage nymphs have lateral plates as well as spiracles. The important characters used in separating the stages of these rat lice may be summarized as follows:

Polyplax spinulosa

Egg—(fig. 1). The egg is about 500–525 micra long by 175–200 micra wide with polygonal, honeycomb pattern on the exochorion. The cap has seven or eight air pores in the center of the cap. The base of the egg is evenly rounded.

First stage nymph—(fig. 2). Length about 0.4 mm. Abdomen elongate with two submedian rows of spines dorsally but not ventrally. Spiracles not surrounded by lateral plates. Posterior part of the abdomen with four very long setae.

Second stage nymph—(fig. 3). Length about 0.6 mm. Abdomen elongate with two submedian rows of spines dorsally and ventrally. Spiracles surrounded by small lateral plates. Seventh and eighth abdominal segments with a pair of long setae on either side, the tip of the abdomen thus having eight long setae.

Third stage nymph—(fig. 4). Length about 0.7–1.1 mm. Abdomen elongate with two submedian rows of spines dorsally and ventrally. Spiracles surrounded by small lateral plates. Sixth abdominal segment with one long and one short setae on either side; seventh and eighth segments with a pair of long setae on either side, the tip of the abdomen thus having 10 long setae.

Female—(fig. 5). Length about 1–1.5 mm. Antenna stout, five-segmented, second segment about as long as wide. Head slightly narrowed anteriorly. Sternal plate approximately five-sided, anterior margin truncated to somewhat pointed. Abdominal segments four to six each with two tergal and two sternal plates, the anterior plate longer than the posterior plate, each plate typically bearing five to seven setae. Lateral plates on segments three to six small, triangular, posterior margin slightly convex with two short subequal setae, the setae on lateral plates seven and eight very long. Posterior margin of abdomen slightly concave.

Male—(fig. 6). Length about 1.1–1.5 mm. Similar to the female, but abdomen with one tergal and one sternal plate each on segments four to six. The third antennal segment has a projecting spur. Posterior margin of abdomen somewhat pointed. Male terminalia with the pseudopenis stout, wedge-shaped, and strongly curved dorso-ventrally, appearing markedly hook-like in lateral view.

Hoplopleura oenomydis

Egg—(fig. 7). The egg exclusive of the holdfast is about 550–600 micra long by about 225–275 micra wide. The cap has nine or ten air pores near the periphery. There is a definite projection at the base of the egg. The exochorion has a reticular appearance in certain lights.

First stage nymph—(fig. 8). Length about 0.4–0.6 mm. Abdomen rather heart-shaped with a double row of six plates dorsally and two long, slender setae at the posterior end of the abdomen. Spiracles absent.

STRUCTURE	HOPLOPLEURA OENOMYDIS	POLYPLAX SPINULOSA
Antenna	More slender, second segment longer than thick, a clasper-like, setose area between segments 4 and 5	Stouter, second segment about as long as thick, no clasper-like setose area present
Sternal plate	Elongate egg-shaped	Subpentagonal
First abdominal sternite	Bell-shaped	Transverse
Lateral plate III*	Deeply emarginate on posterior side, 2 stout spines	Not emarginate on posterior side, with 2 stout spines
Lateral plate IV	Deeply emarginate on posterior side, 1 stout spine, 1 delicate hair	Not emarginate on posterior side, with 2 short, stout spines
Lateral plate V	Deeply emarginate on posterior side, 1 stout spine, 1 delicate hair	Not emarginate on posterior side, with 2 short, stout spines
Lateral plate VI	Deeply emarginate on posterior side, 1 stout spine, 1 delicate hair	Not emarginate on posterior side, with 2 short, stout spines
Second sternite	Extending from lateral plate to lateral plate with 7 to 10 (usually 8 or 9) setae	Not extending from lateral plate to lateral plate, with 4 to 6 setae
Third sternite	Extending from lateral plate to lateral plate, typically with 7 setae, the 4 "paired setae" longer and heavier than the middle three	Not extending from lateral plate to lateral plate, typically with 6 setae
FEMALE: ABDOMINAL SEGMENTS IV–VI	With 3 sclerites on each abdominal segment dorsally and ventrally	With 2 sclerites on each abdominal segment dorsally and ventrally
MALE: ABDOMINAL SEGMENTS IV–VI	With 2 sclerites ventrally and 1 sclerite dorsally on each abnominal segment	With 1 sclerite ventrally and dorsally on each segment

* The lateral plates have been called pleurites or paratergal plates by various authors.

Second stage nymph—(fig. 9). Length about 0.6–1.2 mm. Abdomen heart-shaped, finely granular, entirely without plates, spiracles, or long hairs. There are six minute setae at the posterior end of the abdomen dorsally which can be seen with high magnification of the compound microscope (50 power or above).

Third stage nymph—(fig. 10). Length about 0.6–1.2 mm. Abdomen heart-shaped, finely granular, entirely without plates, spiracles or long hairs. There are six minute setae at the posterior end of the abdomen dorsally and a pair of slightly larger setae on either side just anterior to them. These can be seen with high magnifications of the compound microscope (50 power or above).

Female—(fig. 11). Length about 1.2–2 mm. Antenna stout, five-segmented, second segment longer than wide, a slightly emarginate area between segments four and five. Head more narrowed anteriorly than in *Polyplax*. Sternal plate rather elongate, egg-shaped. Third sternite with two stout spines on each end and three

or more slender setae between. Abdominal segments four to six each with three short tergal and sternal plates, each plate typically bearing six to eight rather slender setae. Lateral plates on segments four to six with spiracles, and with a broad shoulder and a deep, wide emargination on the posterior margin, one large and one minute setae in the emargination. Lateral plates on segments seven and eight much smaller, each bearing two long setae. Posterior margin of abdomen slightly concave.

Male—(fig. 12). Length about 1–2.5 mm. Similar to the female, except that each of abdominal segments four to six has only one tergal plate and two sternal plates. Posterior margin of the abdomen somewhat pointed. Male terminalia with basal plate slightly larger than parameres, arms of the pseudopenis slightly serrulate.

Since it is often more important to identify the adults of these two species of rat lice than the immature stages, the following tabular comparison of the important structures used in distinguishing between adults of these two species has been prepared.

Geographical Distribution. It is probable that the spiny rat louse (*P. spinulosa*) occurs wherever domestic rats (*R. rattus* and *R. norvegicus*) occur in the United States, Puerto Rico, and the Virgin Islands. This species has been found on domestic rats throughout the world and in every state in the United States where sizable collections of rat ectoparasites have been made, as shown in figure 13. The greater number of dots on the map in such states as Georgia and Texas reflect both intensive surveys and the larger number of counties in these areas. In the South, *P. spinulosa* occurs on rats in buildings and in the open, in cities and on farms; in the North, it is more abundant, like its hosts, in buildings in cities.

The tropical rat louse (*H. oenomydis*), on the other hand, appears to be a more recent introduction into the United States as indicated by its occurrence in seaport cities such as Seattle, San Francisco, Galveston, Mobile, Miami, Jacksonville, Savannah, New Bern, and Norfolk (see fig. 14). Its spread inland has been along the main arteries of commerce in such urban areas as Atlanta, Nashville, Montgomery, Kansas City, and Fort Worth where it may have been carried on domestic rats from port cities by trucks or railroads. Its maximum spread inland known to the present writers is Ames, Iowa (Pritchard, 1947), Kansas City, Mo., Nashville, Tenn., and Richmond, Va. The last three records are based on specimens in the Communicable Disease Center collections. It is usually found in the large cities, but is rare or absent in many rural areas in the South. For example, it has not been found in Brooks, Decatur, Grady, or Thomas Counties in Georgia although well-trained entomologists have watched for it carefully over a period of 3 years. While *P. spinulosa* may eventually be found in every state in the Union, the present data indicate that *H. oenomydis,* which was originally described from specimens collected in Africa and the Philippine Islands, may be restricted to the southern part of the United States.

ACKNOWLEDGMENTS

The writers are deeply indebted to many entomologists in the Communicable Disease Center, Atlanta, Ga. or in the various State health departments for their interest and advice during this study, particularly to G. H. Bradley, R. F. Fritz, A. E. Pritchard, N. E. Good, C. O. Mohr, R. B. Eads, L. J. Ogden, R. C. Barnes,

and E. J. Hansens. G. F. Ferris of Stanford University, and C. F. W. Muesebeck of the Bureau of Entomology and Plant Quarantine have read the manuscript and made many constructive criticisms. The plates were drawn by C. J. Stojanovich.

SUMMARY

The paper includes descriptions and drawings of the eggs, three nymphal stages, males and females of the spiny rat louse (*Polyplax spinulosa*) and the tropical rat louse (*Hoplopleura oenomydis*). There are maps showing the distribution of these two species in the United States, the only sucking lice commonly collected from domestic rats during the joint Murine Typhus Control Program of the State health departments and the Communicable Disease Center of the U. S. Public Health Service during the period 1945–1952.

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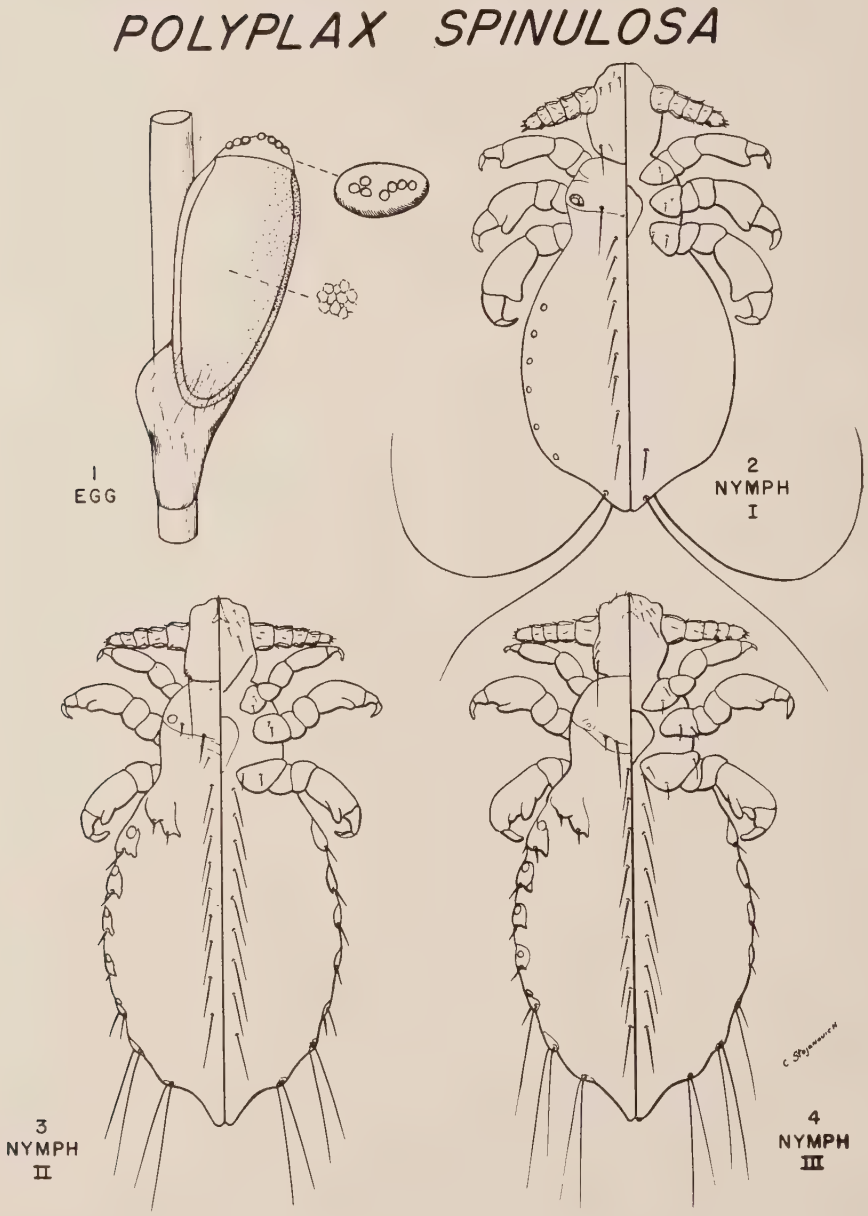


PLATE I. *Polyplax spinulosa*

FIG. 1. Egg.
FIG. 2. First instar nymph, divided dorsal-ventral drawing.
FIG. 3. Second instar nymph, divided dorsal-ventral drawing.
FIG. 4. Third instar nymph, divided dorsal-ventral drawing.

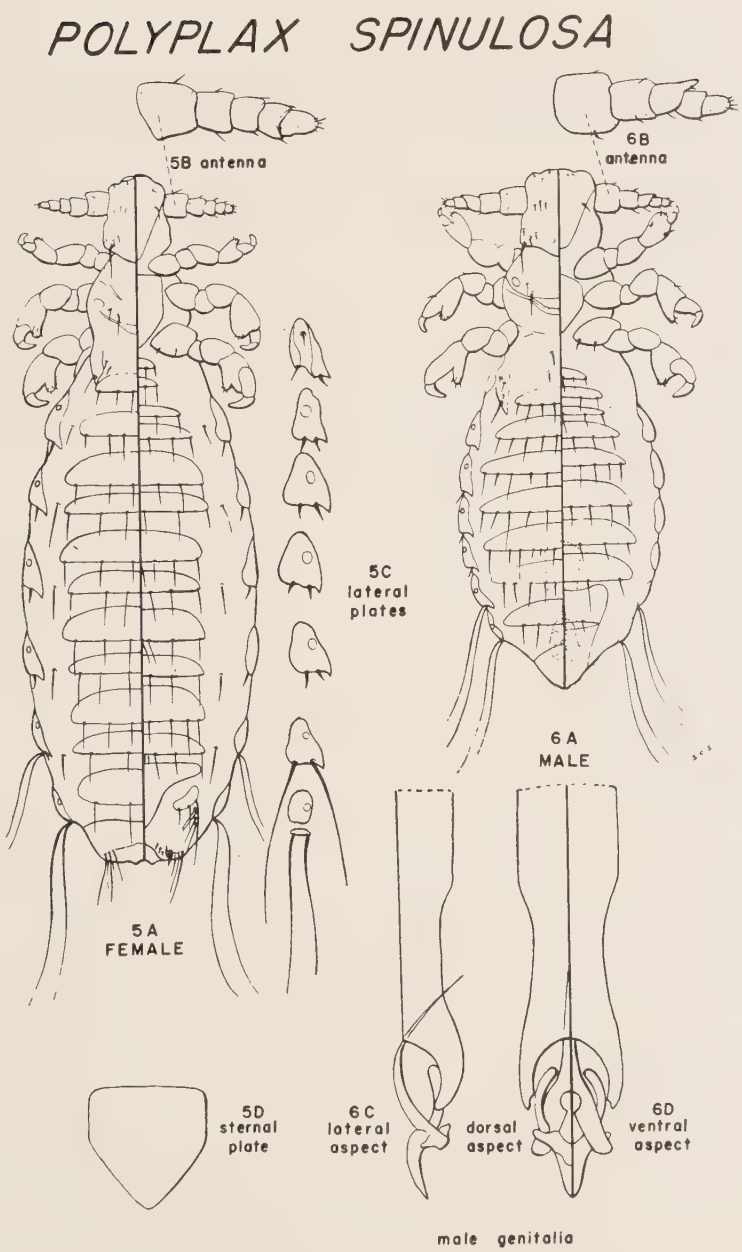


PLATE II. *Polyplax spinulosa*

FIG. 5A. Female, divided dorsal-ventral drawing.
FIG. 5B. Female antenna.
FIG. 5C. Lateral plates.
FIG. 5D. Sternal plate.
FIG. 6A. Male, divided dorsal-ventral drawing.
FIG. 6B. Male antenna.
FIG. 6C. Male genitalia, lateral aspect.
FIG. 6D. Male genitalia, divided dorsal-ventral drawing.

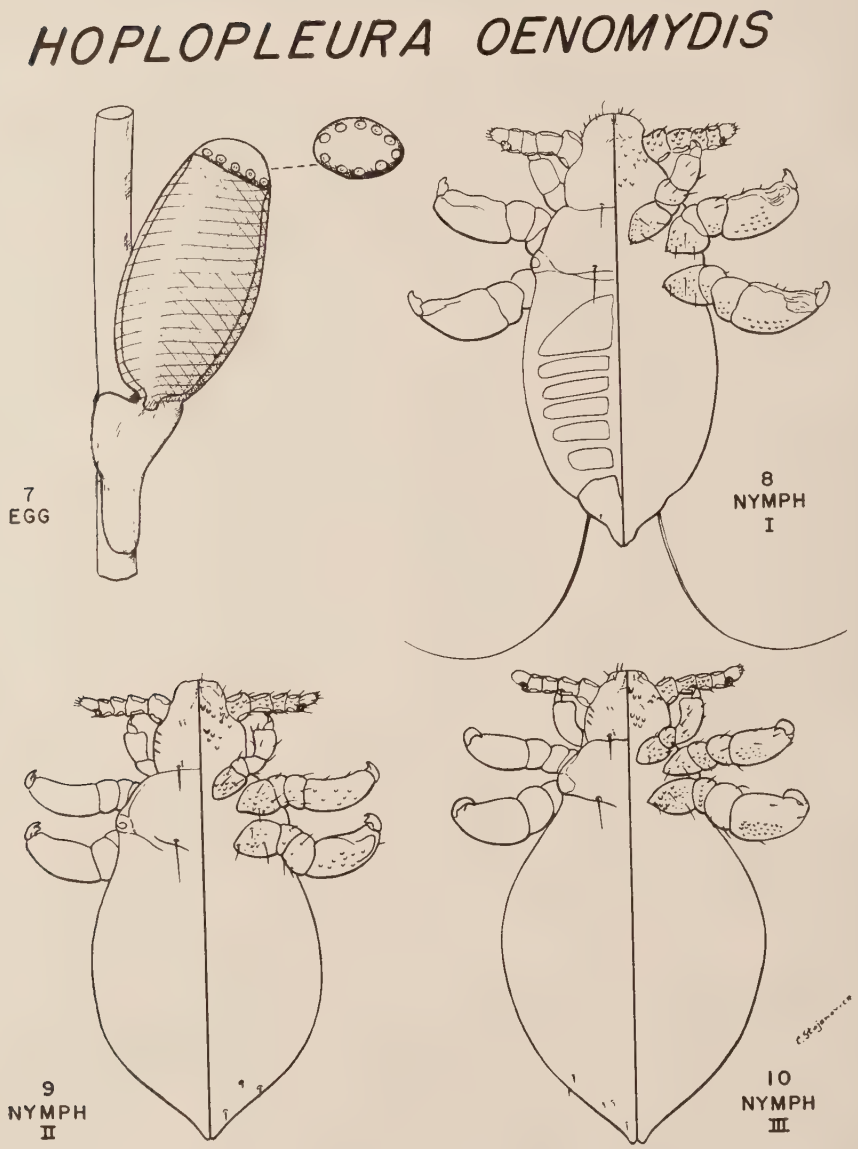


PLATE III. *Hoplopleura oenomydis*

FIG. 7. Egg.
FIG. 8. First instar nymph, divided dorsal-ventral drawing.
FIG. 9. Second instar nymph, divided dorsal-ventral drawing.
FIG. 10. Third instar nymph, divided dorsal-ventral drawing.

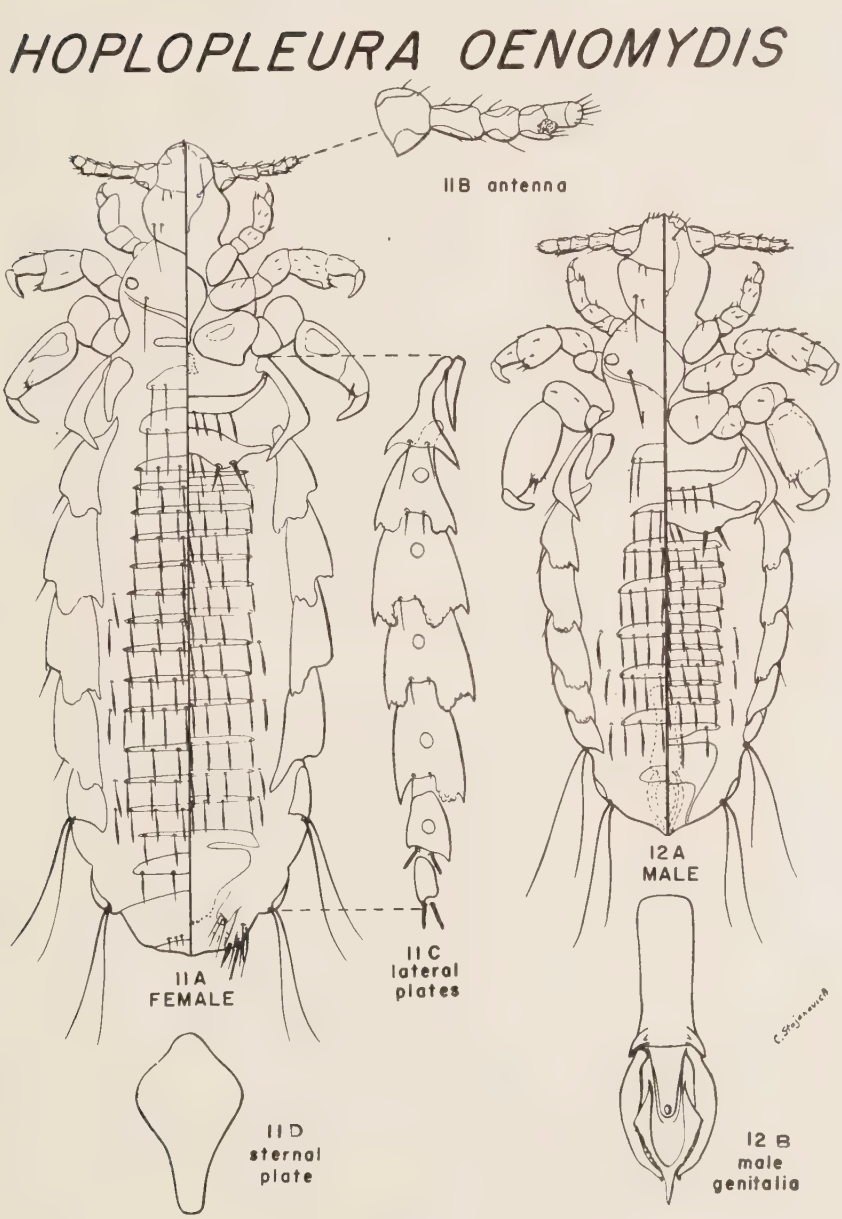
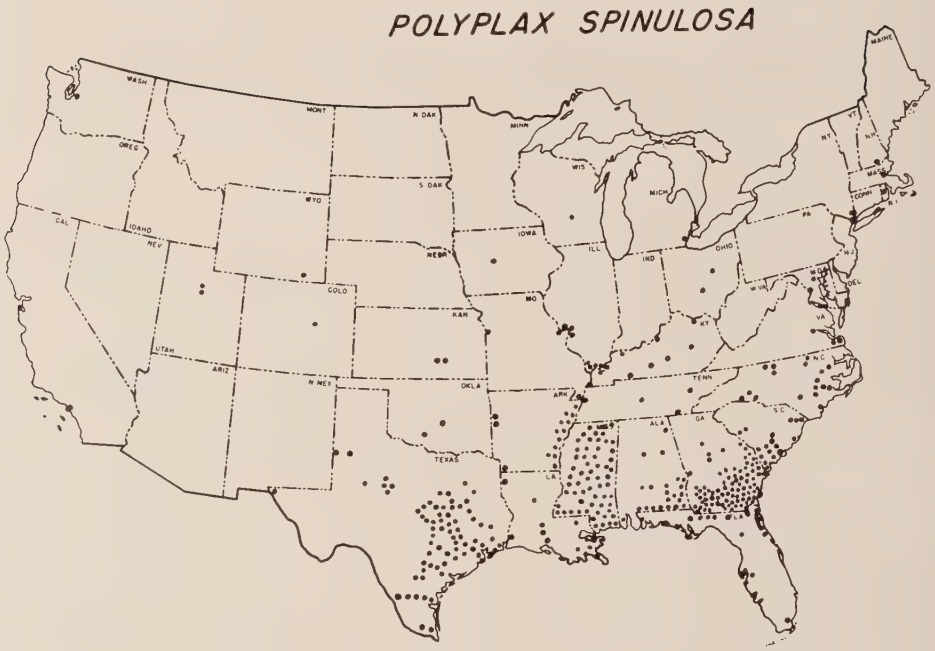


PLATE IV. *Hoplopleura oenomydis*

FIG. 11A. Female, divided dorsal-ventral drawing.
FIG. 11B. Female antenna.
FIG. 11C. Lateral plates.
FIG. 11D. Sternal plate.
FIG. 12A. Male, divided dorsal-ventral drawing.
FIG. 12B. Male genitalia.



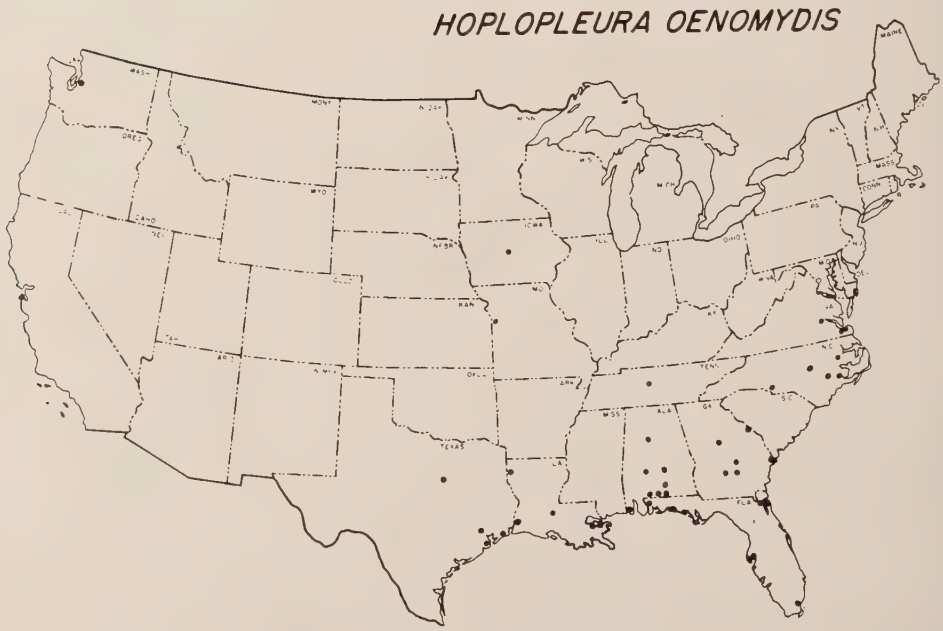


PLATE V

FIG. 13. Distribution map of *Polyplax spinulosa*.
FIG. 14. Distribution map of *Hoplopleura oenomydis*.

ORNITHODOROS ARENICOLOUS SP. NOV. (IXODOIDEA, ARGASIDAE) FROM EGYPTIAN DESERT MAMMAL BURROWS

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An extensive survey of Egyptian mammal burrows from Mersa Matruh on the Western Desert, through the Eastern Desert and Sinai Peninsula, and as far south as Asyut Province, has revealed an obviously rare, undescribed species of *Ornithodoros* tick from widely scattered localities in this area. Adults of the new species are readily distinguished by relatively small size, large cheeks, irregularly and narrowly ridged integument which gives them a wrinkled appearance, short and sparse body hairs, absence of dorsal humps on tarsi, and presence of a small but distinct subapical dorsal protuberance on tarsi II, III, and IV. These distinctive characters readily separate it from *O. tholozani tholozani* (Laboulbène and Mégnin), apparently its closest relative.

Ornithodoros arenicolous sp. nov.

Holotype: Male, engorged (from rearing lot 608) taken as an adult in burrow of hedgehog (*Hemiechinus auritus aegyptius* Fischer) in littoral desert area much disturbed by old military trenches and pill boxes, Ezbet Dawod Farag, Mersa Matruh, Western Desert Governorate, Egypt, 10 September 1952, H. Hoogstraal *legit.* Deposited in United States National Museum, Number 2096.

Allotype: Female, engorged, same data as holotype.

Paratypes: Total 69 ♂♂, 87 ♀♀, 102 nymphs; also 100 unengorged larvae (mounted on slides) and 100 engorged larvae reared from paratype parents in laboratory. 33 ♂♂ and 31 ♀♀ reared from nymphs, 35 nymphs (rearing lots 601 and 602) from hedgehog burrow in side of wadi 50 feet west of that from which holotype was taken, 2 May 1952. 1 ♂, 1 ♀, 1 nymph (rearing lot 609), same burrow as above paratypes, 10 September 1952. 13 ♂♂, 10 ♀♀, 57 nymphs (rearing lot 608), same collecting data as for holotype. 4 ♂♂, 1 ♀ (rearing lot 607), and 1 ♂, 1 ♀ (rearing lot 610), from individual hedgehog burrows in sand dyke surrounding garden, same locality as above, 11 September 1952. 1 ♂ from nest of jird (*Meriones shawi shawi* Duvernoy), Mersa Matruh, 2 May 1952. 6 ♂♂, 13 ♀♀, 2 nymphs, from empty burrow of fat sand-rats (*Psammomys obesus obesus* Cretzschmar) on side of small hill on littoral desert 10 miles east of Mersa Matruh, 1 May 1952. 15 ♂♂, 12 ♀♀, 4 nymphs (rearing lot 606), from four empty burrows of jirds, littoral desert 12 miles east of Mersah Matruh, 11 September 1952. 1 ♂, 1 ♀ (rearing lot 605), from empty burrows of jirds, littoral desert 25 miles east of Mersa Matruh, 11 September 1952. 2 ♂♂, 2 nymphs (rearing lot 604), from empty burrow of jird, littoral desert 40 miles east of Mersa Matruh, 11 September 1952. 2 ♀♀ (rearing lot 603), from empty burrow of fat sand-rat, littoral desert, El Daba, Western

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* The opinions or assertions contained herein are the private ones of the writer and are not to be contstructed as official or reflecting the views of the Navy Department or the Naval Service at large.

Desert Governorate, 11 September 1952. 1 ♀ from old nest of fat sand-rat, littoral desert, El Alamein, Western Desert Governorate, 1 May 1952. 6 ♀♀ from four burrows of gerbils (*Gerbillus gerbillus gerbillus* Olivier), Eastern Desert, barren area above slightly vegetated wadi near entrance of Wadi Digla 2 miles east of Maadi (southern suburb of Cairo), 22 May 1952. 4 ♂♂, 6 ♀♀, 1 nymph, from burrow of Egyptian dab lizard, *Uromastyx aegyptia* (Forskal), same locality and date as preceding.

All specimens collected by Harry Hoogstraal with assistance of persons mentioned in ACKNOWLEDGMENTS.

Paratype specimens deposited in collections of United States National Museum; Fouad I Entomological Society, Cairo; Rocky Mountain Laboratory, Hamilton, Montana; Museum of Comparative Zoology, Harvard University; British Museum (Natural History); Chicago Natural History Museum; Institut Pasteur de l'Iran; Dr. R. B. Heisch, Nairobi; Division of Veterinary Services, Onderstepoort; the writer, and other persons and institutions.

DESCRIPTION

Male (partly engorged): Average measures 3.2 mm. long, 2.1 mm. wide (range 2.6 mm. to 3.6 mm. long, 1.6 mm. to 2.5 mm. wide). *Body* (engorged) with parallel sides, broadly rounded posteriorly, slightly less broadly rounded anteriorly (when unengorged or newly molted the anterior margin is acutely pointed and may form a small anterior knob, also the lateral margin may have one, two, or three concavities); *color* in life (without fresh blood meal) gray with beige legs and cheeks (with fresh blood meal, purple gray). *Dorsal integument* appears wrinkled under low magnification; elevated features consist of narrow, slightly raised ridges forming straight, curved or sinuous lines, and varying between short, unbroken raised lines and rows of adjacent mamillae-like knobs separated by small gaps; ridges completely or incompletely surrounding depressed areas of variable size and shape; ridges on periphery and in center of anterior fourth of dorsum tending towards roughly parallel, longitudinal rows, those elsewhere on dorsum tending towards transverse rows; depressed areas between ridges with uneven, shagreened surface, somewhat larger and shallower on the anterior third, tending towards transversely ovoid and smaller on posterior two-thirds of dorsum, becoming deeper laterally and posteriorly and markedly so on posterior dorsum and on posterior venter; a single very fine and short hair arising from scattered depressed areas, especially noticeable laterally and anteriorly but nowhere becoming locally numerous. (Regularity and degree of ridge and depression characters mentioned above subject to much variation due to differences in extension of body wall between emaciated and engorged state.) Ventral integument posterior of transverse postanal grooves with depressions about twice as deep as anywhere else on body; elsewhere on venter, depressions somewhat shallower and ridges narrower and more regular than on dorsum. *Discs* fairly large, easily discernible by symmetrical arrangement, generally by smoother and more shiny surface, and by more regular, less broken encircling ridges than elsewhere; in addition to paired discs, a median longitudinal row extends the length of posterior half of dorsum. *Lateral margin* structurally undifferentiated; in engorged and partly engorged individuals periphery

is slightly raised externally and slopes internally to a shallow depression delimiting dorsum and periphery (in unengorged individuals periphery forms a wide upturned flange encircling the body and meeting the central dorsum at right angles).

Capitulum moderately large, lying in deep camerostome, not visible from dorsal view (except rarely in grossly engorged or newly-molted individuals). *Cheeks* prominent, in life more yellowish than integument, entirely surrounding and in some positions somewhat overlapping capitulum; consisting of a pair of narrow rugose, elongately triangular lateral sclerites, commencing beside the distal third of the basis capituli and extending to the distal margin of third palpal segment, and of an anterior, ventrally-pointing sclerite (hood); these sclerites heavily ridged and their free ends coarsely serrated. Two or three pairs of rather closely grouped small hairs on anterolateral body surface just dorsad of hood, and a mediolateral pair of very slightly longer hairs on hood itself, but no dense patch or specially long hairs in this area. *Basis capituli* commencing basally level with apex of coxa I or very slightly anterior of this, separated by a deep groove from a fleshly basal area characterized by horizontal ridges, which, in turn, is separated from the intercoxal integument by a deep groove level with the middle of coxa I. *Posthypostomal hairs* arising submedianly and reaching apex of hypostome. *Postpalpal hairs* arising centrally, more widely spaced than posthypostomal pair and reaching only slightly anterior of base of these. In addition, five hairs present basally along each lateral margin of basis capituli, the longest about half as long as the postpalpal hairs, decreasing in length from apical to basal. *Hypostome* measuring about 0.135 mm. long, very slightly concave apically but not notched; denticles on apical three-fifths, 2/2, in a lateral and sublateral file of four larger apical denticles and six smaller basal denticles, the apical two or three transverse rows of these smaller denticles each with an additional third small denticle medially; corona with five or six rows of up to eight hooklets on each half. *Palpi* extending beyond hypostome by length of segment 4 and apical half of segment 3, segments cylindrical, basal three segments progressively shorter than preceding segment but segment 4 about half again as long as segment 3.

Eyes absent. *Spiracular plates* laterad of apex of coxa IV. *Genital aperture* with straight posterior border at level of base of coxa I, anterior border broadly rounded, lying in a lozengal area formed by a deep anterior groove and the interior grooves of the coxal folds; integument just posterior of genital aperture with several parallel rows of narrowly spaced transverse ridges. *Anus* elliptical, situated one third of distance between apex of coxa IV and posterior margin of body; two or three exceedingly small pairs of anterior and posterior hairs on each valve. *Preanal groove* forming a transverse line anterior of anus, extending posterolaterally in a concave arc occupied by contiguous, shagreened discs. *Median postanal groove* with eight to ten pairs of discs extending from anus through transverse postanal groove nearly to posterior margin of body. *Transverse postanal groove* with wide, shagreened anterior and posterior lips which appear minutely, longitudinally striated internally, lips rather widely divided medially by median postanal groove. *Dorsoventral* groove present. *Coxal fold* distinct, with parallel longitudinal rows of small ridges, extending from apex of coxa II to lateral arms of preanal groove, and following this groove to its distal termination. *Supracoxal* fold distinct, extending from cheeks to spiracle.

Legs comparatively short and narrow in diameter; leg I two-fifths as long as body (apex of tarsus to apex of coxa), legs II and III seven-eights as long as IV, leg IV very slightly longer than I. Coxae II, III and IV subequal, contiguous; I larger and separated from II; surfaces weakly resembling body integument except centrally; deep mouth of coxal gland visible just posterior of coxa I. *Tarsus I* with two small submedian dorsal depressions (which in exceptionally distinct instances delimit a median dorsal area faintly suggestive of a hump); tarsal segment gradually expanding dorsoventrally from base to apex and with a more or less acutely retrograde dorsoventral angle delimiting the apical tapered portion and forming a subapical, distally-pointing projection; distal fifth of segment bluntly tapered. *Tarsi II, III* and *IV* with a small subapical dorsal protuberance in the shape of a V on its side; elevation of protuberance variable but in some specimens equalling as much as one-fourth the tarsal width, always distinct except on tarsus II on which it may rarely be very slight; distal tapering equals one-third length of each tarsal segment (longer than on tarsus I and therefore appearing more gradually tapered), length of distal tapering of tarsus II in some specimens varying between that of tarsus I and tarsus III; tarsi ventrally with five pairs of hairs though II and III sometimes have only two to four pairs. *Claws* almost one-fourth length of tarsi; pads small.

Female: Average measures 4.1 mm. long, 2.8 mm. wide (range 3.3 mm. to 4.9 mm. long, 2.2 mm. to 3.7 mm. wide but specimens less than 3.7 mm. by 2.5 mm. rare). Genital aperture straight or forming a slightly concave arc, situated level with apex of coxa II or very slightly anterior of this, lips with fine, narrow longitudinal rugosities; in a number of unengorged specimens a slightly depressed area simulating the male aperture is situated directly anterior of the anterior lip, *i.e.* exactly where it is in the male. Variations in shape and regularity of integumental ridges under different conditions of engorgement and of tarsal protuberances similar to those of male. Except for difference in size and sexual aperture, males and females are alike in morphological characteristics.

Nymph: The *size* of engorged fourth stage nymphs may equal that of any male and exceed that of smaller males. Unengorged nymphal measurements are: first instar, 1.00 mm. long, 0.65 mm. wide; second instar, 1.60 mm. long, 1.00 mm. wide; third instar, 2.00 mm. long, 1.40 mm. wide; fourth instar, 2.70 mm. long, 1.90 mm. wide.

Cheeks of last instar nymphs are narrow, immobile, lateral sclerites almost equalling palpal length; cheeks absent on earlier instars. Mouthparts protected dorsally by a small conical hood with a narrow, blunt apical margin and with two or three pairs of comparatively long hairs dorsally; in fully engorged nymphs, in which the body is much distended, the hood may be forced into a ventrally-pointing position as in adults.

Integumental protuberances are mammillae slightly elevated and separated from each other, dorsally smooth, and laterally with a few smooth rays; arranged mostly in irregular rows on anterior half of dorsum, forming complete or incomplete circles posteriorly and laterally; a few short lines of contiguous, unbroken mammillae forming typically adult raised ridges posteriorly on almost all large specimens; *discs* conspicuous especially in unengorged specimens, with shagreened depressed surface,

surrounded by rim of small, elevated mamillae which are encircled externally by a narrow, almost entirely uninterrupted depression. *Body hairs,* especially laterals, proportionally longer than those of adults and therefore more noticeable.

Tarsal protuberances proportionately reduced; subapical dorsal protuberances very small in small specimens; length of distal tapered area of all tarsi (in most specimens) is about equal to that of tarsus I (not longer than that of tarsus I as in the adult).

Hypostome much more narrowly rounded apically than that of the adult; both files consist of four strong denticles (internal file more medial than in adults); a smaller fifth denticle may be present basally in each file (but small basal denticles of adult are absent); denticles only on apical half of hypostome; corona very weakly developed.

Larva (unengorged): Length (excluding mouthparts) about 0.52 mm., width about 0.42 mm.; outline either circular or with sides bulging and with posterior fourth distinctly narrowed. *Integument* with wide, raised striations forming intricate curved, angular, and whorled patterns; integument between coxae thickened and marked by close, wavy, longitudinal striations which slightly converge basally. *Body hairs* dorsally numbering 13 pairs; three long posterior pairs of which the external pair is slightly submarginal and the others are marginal, six equidistantly spaced internal pairs paralleling the lateral margin, two sublateral pairs one of which is about level with coxa I and the other with coxa II, and two submedian pairs one of which is on the anterior margin and the other is about level with coxa II, all these hairs more or less distinctly frayed; ventral hairs numbering six (rarely seven) pairs consisting of three anterior pairs each of which is opposite a coxa and three (rarely four) posterior pairs in a line beside the anus; also ventrally a pair on the anus and on each coxa. *Legs* robust, length equalling body width; coxae slightly separated, I and II quadrangular, III triangular, decreasing in size from I to III, each with a small hair near anterior and posterior margin; trochanters each with a fairly long hair distally on anterior margin; tarsus I with three pairs of short ventral hairs and four pairs of long dorsal hairs, dorsal margin straight on anterior three-fifths, truncate beyond Haller's organ, distal two-fifths gradually tapered. *Anus* from a third to half distance between coxa III and posterior body margin; rounded, with a single, long, fine hair arising from anterior half of each valve. *Capitulum* arising ventrally when withdrawn, anteriorly when extended on basal eversible tube; basis capituli about twice as wide as long, with convex lateral and basal margins, bearing a pair of posthypostomal hairs about three-fourths as long as hypostome, and a pair of postpalpal hairs about three-fourth as long as posthypostomals. *Hypostome* measures 0.06 mm. long, lateral margins parallel, apically rounded, not notched; with a pair of sublateral and submedian files each with five denticles the basal and apical of which are smallest, internal file denticles smaller than externals; denticles confined to apical half of hypostome; corona with a few irregular, scattered, weak hooklets or none. *Palpi* broadly cylindrical, reaching apex of hypostome when in normal, partially telescoped position; fourth segment with seven long, stout setae apically.

Larva engorged measures about 0.82 long, 0.65 mm. wide.

Egg diameter 0.375 mm.

RELATED SPECIES

O. arenicolous sp. nov. is related to the *tholozani* group as defined by Desportes and Campana (1946). It is smaller than *O. tholozani tholozani* (Laboulbène and Mégnin) 1882 of Egypt, Palestine, Syria, Iran, Iraq, and the Caucasus, and has much finer, more jagged, and more irregular integumental elevations. The hood of *tholozani* is a knob fleshier than the cheeks, the hood of the new species is no thicker than the cheeks. In *tholozani* the cheeks are comparatively much shorter, extending only from the distal half of the first palpal segment to the distal margin of segment II, in the new species they commence beside the distal third of the basis capituli and extend to the distal margin of palpal segment III. The conspicuous concentration of fairly long hairs on the anterior surface of *tholozani* is absent in the new species. *Tholozani* usually has a conspicuous indented mediodorsal depression on tarsus I, this is absent or very faint in the new species. The basal hypostomal denticles, nine to twelve rows of six or seven files in *tholozani,* are reduced to six rows of two files plus one to three additional internal pairs in the new species. The seven to nine pairs of distinctly visible anal hairs of *tholozani* are reduced to six or fewer pairs of exceedingly small hairs in the new species.

The other subspecies which form the *tholozani* group, *O. tholozani crossi* Brumpt of Punjab, *O. tholozani pavlovskyi* Desportes and Campana of central Asia, and *O. tholozani persepoliensis* Delpy (1947) of Iran, are even more different from *O. arenicolous* sp. nov. than the typical subspecies, and are geographically more distant. For a review of the much-mooted *O. papillipes* Birula in relation to this group, see Desportes and Campana (1946).

A Russian and Iranian species, *O. tartakovskyi* Olenev, is similar to *O. arenicolous* in size, presence of cheeks, and intricate integumental ridges. *O. tartakovskyi* differs distinctly, however, in formation of integumental ridges, absence of tarsal subapical dorsal protuberances, proportion of tarsal distal tapered area, presence of numerous hairs in the camerostome, posthypostomal hairs extending only half length of hypostome, many more basal small hypostomal denticles, and numerous other characters.

Other African and Asiatic species in which cheeks are well developed have such clearly mammillated integument that no confusion arises in separating them from the new species.

HOSTS

Egyptian hedgehog (*Hemiechinus auritus aegyptius* Fischer), fat sand-rat (*Psammomys obesus obesus* Cretzschmar), jird (*Meriones shawi shawi* Duvernoy), and gerbil (*Gerbillus gerbillus gerbillus* Olivier). Host names of Egyptian mammals, which are in a very unsettled taxonomic state, as here used follow Ellerman and Morrison-Scott (1951).

Specimens have also been taken from a burrow of the Egyptian dab lizard, *Uromastyx aegyptia* (Forskal). These showed no evidence of a recent blood meal and so it cannot be stated whether they had fed on a lizard or a rodent which might have sought refuge in the burrow.

BIOLOGY

Ornithodoros arenicolous sp. nov. occurs in small mammal and large lizard burrows in the littoral area of the Western Desert and in the Eastern Desert near the

Nile Valley. It appears to be absent from most animal burrows throughout this range, but occurs in varying degrees of frequency in localized areas. No known factors can be applied to explain its presence in some localities and absence in other similar areas.

Burrows from which this tick has been taken have all been dry. They have contained no trace of stored, decaying, fleshy vegetation, and the sand in them has been in separate, loose particles uncolored by dampness. The sand in each instance has been mixed with a greater or lesser proportion of grey or light yellow clay. Western Desert collections are from a littoral region of higher atmospheric humidity than that of the inland desert. The Eastern Desert collecting site, in spite of its proximity to the Nile Valley, does not appreciably differ from inland "moderate-desert" conditions, *i.e.* situations in which sparse, scattered flora and fauna occur as opposed to "extreme desert" where plants and animals are totally absent.

As indicated in the list of paratypes, hedgehogs in the Mersa Matruh area may support large populations of this tick. Up to 99 individuals were found in a single hedgehog burrow. Smaller rodent burrows are inhabited by fewer numbers, nine individuals being the maximum number found in a single rodent burrow.

This species is associated with smaller numbers of *Ornithodoros erraticus* (Lucas) in Western Desert hedgehog burrows and larger numbers of *O. erraticus* in rodent burrows. Another species which we find in Egyptian mammal burrows, *O. delanoëi delanoëi* Roubaud and Colas-Belcour, has not been found with *O. arenicolous* sp. nov. The absence of the new species from hedgehog and rodent burrows in dry mounds in the cultivated Nile Delta and Valley, in which *O. erraticus* flourishes and *O. d. delanoëi* is sometimes found, is striking. *O. t. tholozani* was once taken alone in a Western Desert *Psammomys* burrow in April and when the same burrow was examined later in May several specimens only of *O. arenicolous* sp. nov. were found in it.

LIFE HISTORY

The following life history data, obtained at Cairo between July and the last of December, 1952, are based on laboratory rearings under conditions approximate to temperature and humidity in the tick's natural habitat. More specialized studies will be undertaken during the coming season.

Egg batches, laid on the surface of sand or the side of tubes, consist of 16 to 90 eggs. Females remain "brooding" over the eggs until larvae emerge. Individual females have laid separate egg batches from 32 to 41 days apart—without having fed in the adult stage. Ten selected egg batches, containing about 500 eggs which produced vigorous progeny among with the death rate was almost nil, hatched in from 15 to 25 days, average 18.4 days, from late July to early October. Eight other batches were discarded from the study series when the progeny showed a high death rate.

In the following stages, all individuals from four to seven days after molting were fed on hairless baby white mice. The duration of immature stages was: larvae, 16 to 33 days, average 22.2 days; first instar nymphs, 17 to 25 days, average 19 days; second instar nymphs, 11 to 50 days, average 25.3 days; third instar nymphs which molted to adult males, 15 to 54 days, average 38.8 days; third instar nymphs which molted to fourth instar nymphs, 27 to 29 days, average 28 days. In

addition many third instar nymphs have not yet molted after having remained in that stage for over 90 days. Only males have emerged from third instar molts. At the time of writing, no females have emerged from F_1 rearings and no fourth stage nymphs have molted.

Certain other life history data, intimately associated with feeding, are presented below.

FEEDING

Immature stages in the above-mentioned ten selected lots four to seven days after molting were offered hairless baby white mice. All feeding was done in full daylight, little or no hesitancy over feeding was encountered, and the mortality rate was almost nil.

Invariably all larvae rushed to the host when placed with it. They became engorged in 15 or 16 minutes (rarely up to 20 minutes), and dropped off the mouse immediately afterwards.

Individuals in the four nymphal instars commenced to feed within a few minutes to several hours after being placed with the host. Most nymphs attached and commenced feeding within half an hour. Those which had not attached were left overnight and almost all were engorged the next morning. Duration of feeding in all daytime observations was 15 to 20 minutes for the first three instars, 35 to 40 minutes for the fourth instar.

Females have completed engorgement on hairless baby white mice in 45 minutes; on hedgehogs in 45 to 80 minutes, average on hedgehogs 70 minutes. Casual observation has shown no difference in degree of engorgement on these two hosts. Males have completed engorgement on baby mice in from 40 to 55 minutes, average 45 minutes; on hedgehogs in from 30 to 80 minutes, average 57 minutes.

Attempts at feeding "wild-caught" specimens have been particularly interesting. Individuals used for life history studies consisted of 10 pairs, obtained as large nymphs from hedgehog burrows, which molted and were paired soon after collection; 8 pairs of adults from hedgehog burrows, and 11 pairs of adults from various rodent burrow as listed for paratypes. Originally 30 pairs obtained from hedgehog burrows as nymphs or adults were observed for feeding, subsequently these were reduced to the above mentioned 18 pairs to provide material for morphological study and to be able to spend more time with each lot.

Of the 18 pairs from hedgehog burrows, only a single male has fed on hairless baby white mice or on immobilized adult mice which have been offered them on at least six occasions in the past five months. Of seven males offered hedgehogs, only four fed. Yet vigorous progeny have been obtained from females confined with these males, whether the males had fed or not.

Only two females in the same 18 lots from hedgehog burrows have fed on baby or adult mice although they have been offered as for males. Eight females, obtained as adults from hedgehog burrows, which have refused to feed in the laboratory, have laid egg batches which resulted in vigorous progeny and two of them have laid two batches. These females may have fed before capture. Three of the ten pairs obtained as large nymphs from hedgehog burrows, and which we are certain *have never fed in the adult stage*, have laid single egg batches yielding vigorous progeny; three others of the same ten, which also have not fed as adults, have laid egg batches

which gave rise to weak individuals which died in the larval or early nymphal stages; and the other four which have not fed as adults have laid no eggs.

When this unique situation became apparent to us, we offered these same females immobile hedgehogs with shaven undersides. All but two of the 18 females (16 of which had heretofore refused to feed on mice) readily fed on the hedgehogs and reached full engorgement. (No egg laying has followed, possibly because of the lateness of the season when these experiments were done.)

In spite of the parents' apparent reluctance to feed on mice, their progeny have shown no hesitation to do so, as already described.

A single pair of adults, obtained as nymphs from the same hedgehog burrows, which were sent to Dr. G. E. Davis at the Rocky Mountain Laboratory, have readily fed on immobilized white mice and have produced F_1 progeny and a second egg batch. Female F_1 progeny, which we have not yet obtained in Cairo under approximately natural conditions, have been obtained from this pair by Dr. Davis, who has maintained the specimens under regulated temperature and humidity conditions.

In contrast to the ticks taken from hedgehog burrows, the eleven pairs from rodent burrows have readily fed both on baby and adult mice. More detailed observations have not yet been done with these specimens.

DISCUSSION

Ornithodoros arenicolous sp. nov. falls into the first feeding and molting pattern, *i.e.* alternate feeding and molting from larval to adult stage, outlined by Davis (1952) for this genus. The phenomenon of egg laying (with or) without feeding in the adult stage, is, however, unique in this genus.

A comparison of feeding results among individuals taken as large nymphs or as adults from hedgehog burrows with individuals taken from rodent burrows is strongly suggestive of a predilection for hedgehog (Order Insectivora) blood once these ticks have reached an advanced nymphal stage in hedgehog burrows. That this apparent preference does not carry over from "hedgehog-habituated" adults to their progeny is amply indicated.

SUMMARY

1. *Ornithodoros arenicolous* sp. nov. is described as a small member of the *O. tholozani* group.

2. This species is of rare occurrence in localized areas in hedgehog, rodent, and large lizard burrows in the littoral area of the Western Desert and in the Eastern Desert of Egypt.

3. The life cycle is of the alternate feeding and molting type. One and possibly two generations a year occur. Small egg batches are laid. To date, only males have developed from third instar nymphs, others which have molted to fourth instar nymphs have not molted again after as long as three months.

4. Feeding is rapid, 15 to 20 minutes for larvae and first to third instar nymphs and 40 minutes for fourth instar nymphs. Adults reach engorgement in from 30 to 80 minutes, but usually require a longer period for satiation on hedgehogs than they do on baby mice.

5. A phenomenon unique for this genus, in which a vigorous F_1 generation may result from parents which (have or) have not fed in the adult stage, is described.

ACKNOWLEDGMENTS

Persons who assisted in making one or several of the collections of this species were Abdel Aziz Salah, Dr. Kamal Wassif, Makram Kaiser, Sayed Mitwally, Sobhy Gaber, and Ibrahim Soliman Khetr. I am indebted to Sobhy Gaber for careful assistance with the life cycle studies and to Glen M. Kohls for reviewing the manuscript.

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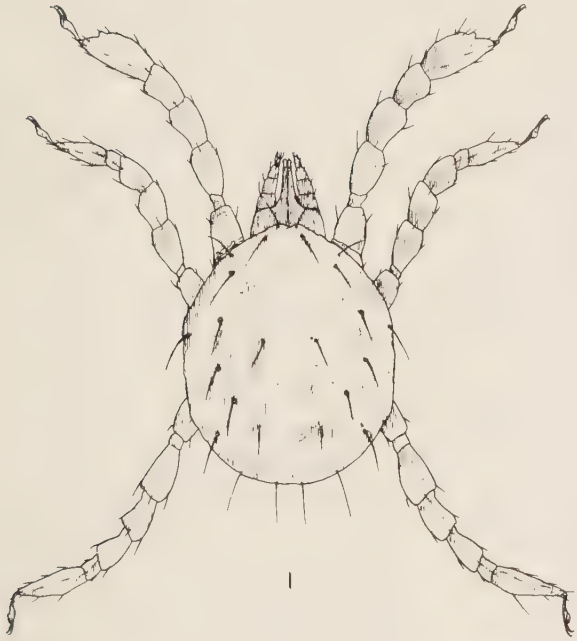
Explanation of Plates

Ornithodoros arenicolous sp. nov.

Fig. 1. Larva; unengorged; integumental striations not shown; dorsal.
Fig. 2. Larva; unengorged; integumental striations shown; ventral.
Fig. 3. Larva; hypostome, palp, and basis capituli; ventral.
Fig. 4. Nymph; first instar; slightly engorged; dorsal.
Fig. 5. Nymph; first instar; slightly engorged; ventral.
Fig. 6. Female; hypostome, palp, and basis capituli; ventral.
Fig. 7. Nymph; first instar; tarsi I to IV; lateral.
Fig. 8. Female; tarsi I to IV; lateral.
Fig. 9. Male; region of genital aperture; ventral.
Fig. 10. Female; partly engorged; lateral.
Fig. 11. Female; partly engorged; dorsal.
Fig. 12. Female; partly engorged; ventral.

PLATE I



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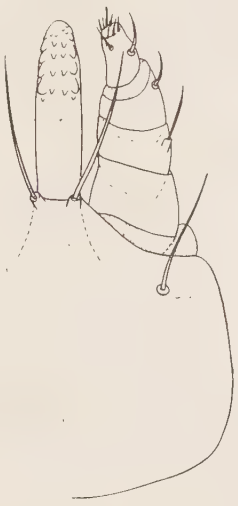

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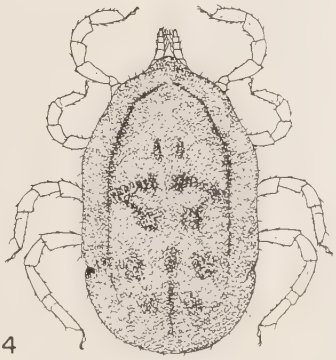

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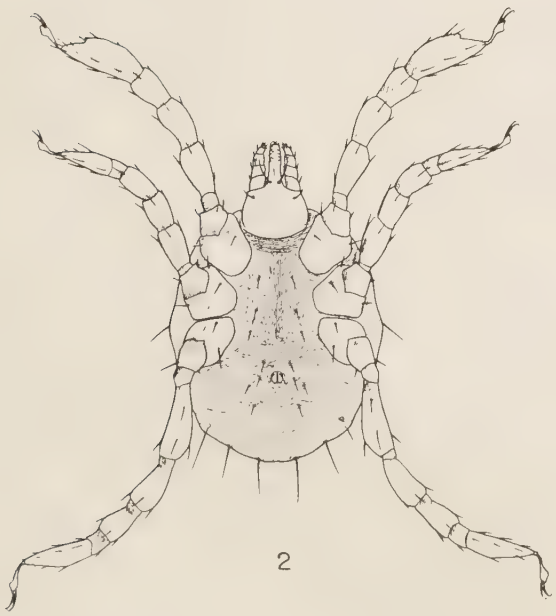

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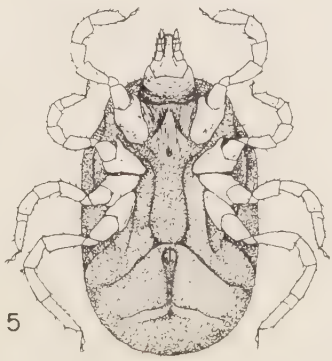

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PLATE II

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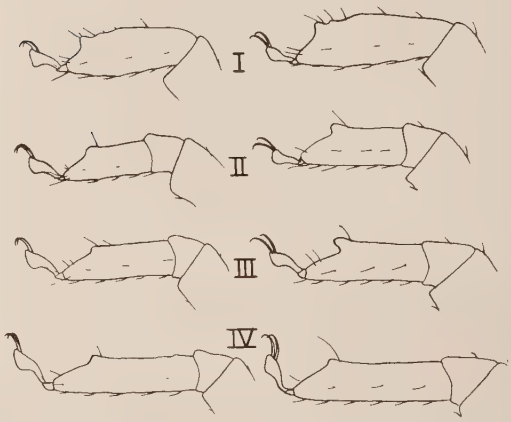

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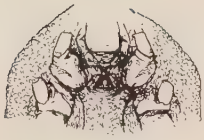

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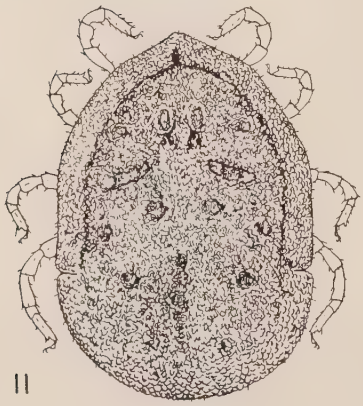

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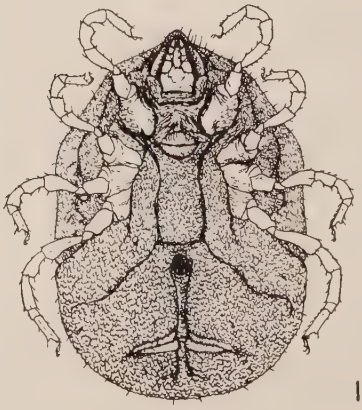

12

LONGEVITY OF SCHISTOSOMA HEMATOBIUM AND SCHISTOSOMA MANSONI: OBSERVATIONS BASED ON A CASE

D. A. Berberian, M.D.,[1] H. O. Paquin, Jr., M.D.,[2*] and A. Fantauzzi, M.D.[2]

There is relatively little known concerning the longevity of schistosomes in the human host since the majority of infections occur in endemic areas where reinfection cannot be eliminated. However, the sporadic imported cases which are observed in a country where the disease is not endemic, furnish information about the survival of the parasites in a specific host. The following authenticated history of prolonged survival of living parasites in an immigrant from Yemen, Arabia, is presented to provide information on the life span of the adult stages of *Schistosoma hematobium* and *S. mansoni* in man.

Case history. Mr. H. H., a native of Yemen, Arabia, immigrated to the United States 27 years ago, at the age of 23. He settled in northern New York State and since then has

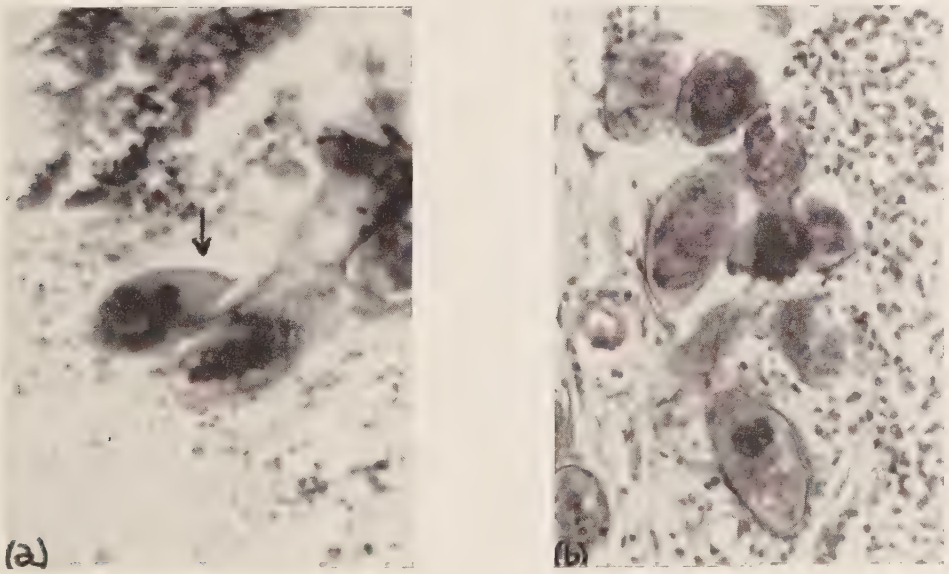

Fig. 1. (a) Ova of *Schistosoma hematobium* in wall of appendix. (b) Ova of *Schistosoma mansoni* in wall of appendix.

not traveled either within the United States or abroad. His only childhood illness occurred at the age of 8 or 10 when he was cauterized over the abdomen for a pain in the epigastrium, coincidental with a roundworm infection. He recalls passing worms through the nostrils at that time, and since then has been in good health. He has no history of blood in the urine, bleeding after micturition, blood tinged stools, dysentery or diarrhoea.

His first hospitalization in this country occurred on February 4, 1951 for the treatment of a "virus pneumonia". He was again hospitalized in May for a pain of one week's duration around the umbilicus. A complete G.I. series was done and x-ray findings suggested chronic duodenal ulcer, enteritis, colitis and probable chronic appendicitis. The patient was discharged on June 3rd and readmitted the following day for an appendectomy, which was performed on June 5, 1951.

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Macroscopic examination of the appendix revealed a grossly normal appendix measuring 6×0.5 cm. Microscopic examination revealed the presence of large numbers of schistosome ova, mostly those of *Schistosoma mansoni* with occasional ova of *S. hematobium* scattered here and there (Fig. 1) in the granulomatous tissue in the wall of appendix. In view of this finding a more thorough examination was made of the patient's stools and urine, which revealed ova of *S. hematobium* in the urinary sediment and ova of *S. mansoni* in the stools. The ova were not numerous and repeated trials failed to induce hatching.

A more thorough examination of the patient was made in an effort to detect foci of abnormal localization. Radiography of the skull and chest were negative for abnormal findings. Proctoscopic examination showed the presence of small hemorrhoids, but there were no ulcers. The hemorrhoids were removed and sections showed numerous calcified ova of *S. mansoni.* Cystoscopic examination failed to reveal gross macroscopic pathology. Urine cultures were sterile with the intermittent appearance of pus cells. On certain days pus cells were very few, and at such times ova were not found in the urine sediment. At other times there were numerous pus cells and squamous epithelial cells with occasional ova of *S. hematobium,* some of which were deformed. A repeat examination of urine and stools on January 10, 1952 revealed the presence of degenerated ova of *S. mansoni* and *S. hematobium* in the urine and of *S. mansoni* in the stools.

A careful inquiry about the possible source of infection revealed that as a child the patient frequently waded or swam in cisterns of rain water impounded for irrigation and domestic use. He said that he never swam in these places after he was 8 years old, since at that age he had to work with his father.

DISCUSSION

Information on the longevity of schistosomes is scarce. Christopherson (1924) cited the case of a British zoologist who acquired vesical bilharzia in 1878 while in South Africa and who, 28 years after his return to England, passed viable ova in the urine. Fourteen years later he was examined and no ova were found in his urine. Fairley (1931) cited another case of vesical bilharzia acquired in South Africa; this patient, after 29 years of uninterrupted residence in England, died of widespread carcinomatosis of the bladder. His urine contained many eggs of *Schistosoma hematobium* which failed to hatch. At autopsy sections from the base and neck of the bladder contained many schistosome ova. Recently Wallerstein (1949) reported a case of *S. mansoni* infection in a 32 year old Puerto Rican woman who was admitted to the hospital for gall stones. This woman had continuously resided in New York City and its immediate environs for 26 years. Because of a rectal bleeding she was sigmoidoscoped prior to cholecystectomy. A pedunculated polyp observed 5 inches from the anus was removed and on biopsy was diagnosed as precancerous. The polyp also contained many eggs of *S. mansoni.* Repeated stool examinations were negative for ova.

Schistosoma mansoni infection is relatively rare in Asia. The only autochtonous cases have been found in Arabia, Yemen (Craig and Faust, 1951) and in Aden (Greval, 1922). Malchi (1924) reported 2 cases of *S. mansoni* infection in Palestine, both in immigrants from Yemen. Sarnelli (1935) reported that vesical schistosomiasis was very common in males of all ages in San'a, Yemen (elevation—7200 ft.), and the neighboring villages on the plateau. Petrie (1939) reported that schistosomiasis was common in Yemen, but failed to specify the type of the disease. Recently Kuntz (1952) reported on a preliminary survey conducted in the Kingdom of Yemen by the United States Naval Research Unit No. 3 of Cairo, Egypt and confirmed earlier indefinite reports that both *S. hematobium* and *S. mansoni* occurred in that country. They collected *Biomphalaria boissyi arabica* from open ablution basins in mosques at Ta'izz and found them heavily infected with *S. mansoni.* This was a first report showing a relationship between religious prac-

tices and schistosoma infection. A cursory survey of open cisterns so common on the San'a plateau revealed the presence of both *Bulinus contortus* and *Biomphalaria boissyi arabica.*

The Yemenite referred to in this paper undoubtedly acquired his infection during his childhood in San'a. Twenty-six years after immigration into the United States and possibly 40 years after infection, he is still passing the ova of both species in stools and urine, indicating that he is harboring living adult schistosomes of both species in his body. The case is also striking in that typical signs and symptoms of both infections have been inapparent throughout this long period, indicating that if initial infection is not heavy and reinfection does not occur, specific symptoms of the disease may not appear.

SUMMARY

A case of *Schistosoma mansoni* and *S. hematobium* infection is reported in a 49 year old Yemenite male, who left the endemic area at the age of 23 and has since resided in Mechanicville, N. Y. Evidence is presented that this patient, 26 years after leaving the endemic focus of infection, has adult flukes of both species in his body, alive and ovulating. The case history shows that the infection was undoubtedly acquired in childhood and is possibly of 40 years duration.

Absence of signs and symptoms directly referrable to his double infection is noted.

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LECITHOCHIRIUM LYCODONTIS N. SP., TREMATODE FROM THE MORAY EEL OF THE NEW HEBRIDES[1]

B. J. Myers[2] and R. W. Wolfgang[3]

Ten trematodes belonging to the genus *Lecithochirium* were included in a collection of helminths from the South Pacific forwarded to the Institute of Parasitology by Dr. Robert E. Kuntz, United States Medical Research Unit No. 3, Cairo, Egypt, for examination and identification. Comparison of these specimens with described species of the genus indicates that the present material constitutes a new species. The authors wish to thank Dr. H. W. Manter for his advice and the loan of specimens.

LECITHOCHIRIUM LYCODONTIS N. SP.

Specific Diagnosis: Body elongate and cylindrical, the posterior portion with an ecsoma which can be protruded or retracted into the body. The body length, excluding the ecsoma, ranges from 3.0 to 4.3 mm., ecsoma when extruded measures 0.15 to 1.3 mm. Maximum width 0.69 to 0.90 mm. The cuticula is rugose and wart-like papillae appear on the folds. Oral sucker subterminal 0.24 to 0.33 mm. in diameter. Acetabulum very large and prominent, 0.48 to 0.63 mm. in diameter. Sucker ratio approximately 1 : 2. Pre-acetabular region 0.69 to 1.0 mm. long. Pharynx muscular and globular with a diameter of 0.135 mm. Prepharynx and esophagus apparently lacking; ceca long and may extend into the ecsoma. Presomatic pit is lacking. Genital pore median, anterior of acetabulum. Testes side by side immediately posterior to acetabulum. Seminal vesicle bipartite, the anterior portion almost spherical and thick-walled, posterior portion sac-like and thin-walled. Sinus sac thick-walled, 0.315 mm. in length and 0.375 mm. in diameter. Prostatic vesicle small, lying inside the sinus sac. Genital sinus very broad and can be everted or prolapsed. When everted it appears wrinkled or convoluted as shown in the Figure. When retracted, it has a transverse slit-like cavity. Ovary smooth, ovoid, to the left or right and about midway between acetabulum and base of ecsoma. Vitelline lobes thick, variable, often masking the ovary. Uterus does not enter ecsoma; the terminal portion forms a muscular metraterm. Eggs thin-shelled, 10 to 13 μ by 6 to 7 μ.

The specific name *lycodontis* is for the host.

Host: Moray Eel, *Lycondontis* sp.

Location: Cecum, large intestine.

Locality: Espiritu Santo, New Hebrides.

Number: Ten specimens were collected from two hosts.

Type Specimen: Deposited at the National Museum, Washington, D. C., U. S. A. No. 47902.

DISCUSSION

Manter (1947) discussed the morphological differences between *Lecithochirium* and *Sterrhurus* and commented on the work of Jones (1943) and Crowcroft (1946). The authors agree with Manter in considering that the only reliable character separating these two genera is the bladder within the muscular pouch or sinus sac, which in *Lecithochirium* is a portion of the pars prostatica while in *Sterrhurus* the bladder is derived from a portion of the ejaculatory duct (Crowcroft, 1946). In *L. lycondontis* the bladder is derived from the pars prostatica. *Lecithochirium lycodontis* differs from other species in:

(1) the rugose ventral cuticula with wart-like papillae on the folds,

(2) the bipartite seminal vesicle, the anterior portion of which is thick-walled and almost spherical; the posterior portion is thin-walled and sac-like,

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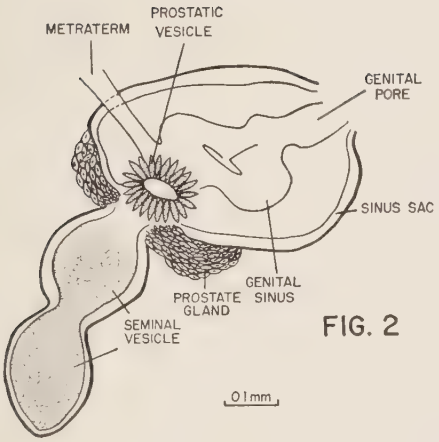

FIG. 2

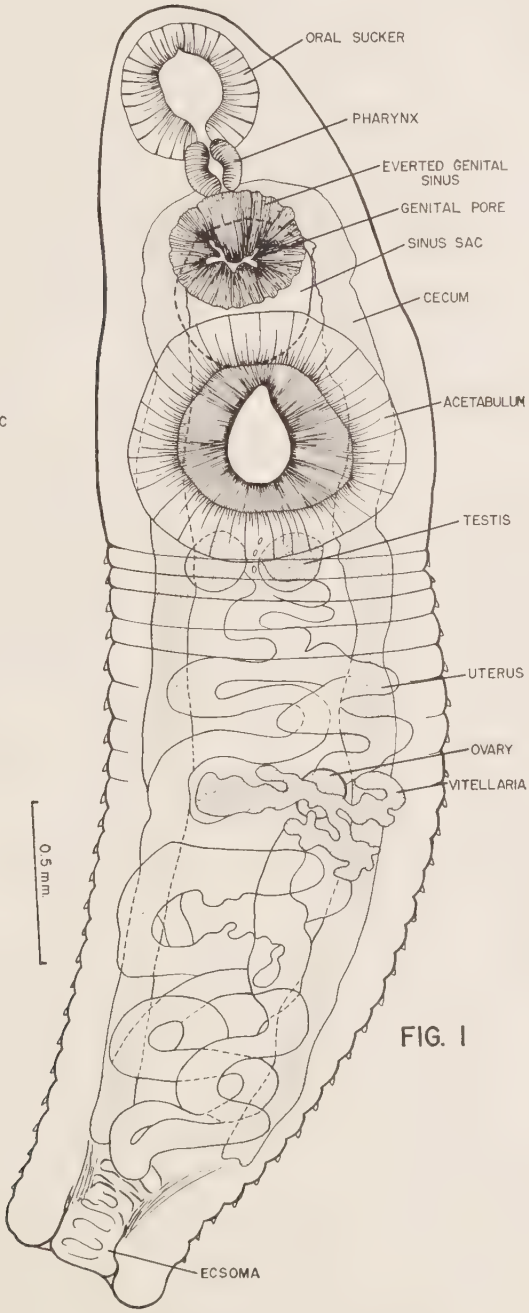

FIG. 1

FIG. 1. *Lecithochirium lycodontis*, whole mount from ventral aspect.
FIG. 2. *L. lycodontis*, terminal genital organs.

(3) the broad, muscular genital sinus which may be everted or prolapsed,
(4) the large acetabulum.

L. lycondontis resembles other known species in:

(1) the bladder is derived from the pars prostatica,
(2) undivided short, stout lobes of vitellaria,
(3) the arrangement of the reproductive organs.

L. lycodontis displays the morphological characters described above as well as having an abnormally large acetabulum, and a very compact prostatic vesicle composed of granular cells. These characteristics may be sufficient for establishment of a new genus. However, due to inadequate material for comparative purposes distinction only at the species level seems advisable.

The sinus sac varied so little in the ten specimens examined that the measurement given may be considered as valid for all of them regardless of the contraction of individual worms. Contractions due to fixation resulted in a considerable variability in the body proportions as well as in the shape of the suckers.

Manter (1947) suggested that the loose, open nature of the sinus sac or the character of the male vesicle within the sac could be used as characteristic of the *Lecithochirium*. A redescription and redefinition of the generic characteristics seems necessary to establish a definite separation of *Lecithochirium* and *Sterrhurus* which are closely related.

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EXPERIMENTAL RESULTS ON POSSIBLE ARTHROPOD TRANSMISSION OF TOXOPLASMOSIS

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The most commonly recognized manifestation of toxoplasmosis in humans is a severe disease of the central nervous system in newborn infants. Neonatal toxoplasmosis is definitely of congenital origin; yet the mother shows no history of illness preceding delivery. Thus, in adults, *Toxoplasma* infection may be entirely asymptomatic, and the occurrence of the disease in infants is due to the unfortunate circumstance that the mother acquires the infection during the gestation period. On the basis of survey data, it appears that *Toxoplasma* infection is widespread in man (Feldman and Sabin, 1949) and animals (Jacobs, Melton, and Jones, 1952). The mode of transmission, however, is still a matter of conjecture. The organism, in the stage in which we know it, is not resistant to environmental conditions outside the host, and there is little evidence that the contaminative method could serve for its spread. Transmission by bloodsucking arthropods has been postulated by several investigators, and a few experimental attempts have been made to test various arthropods as vectors. References to the pertinent literature are included in the bibliographies of Weyer (1951), Piekarski (1950), Blanc, Bruneau, and Chabaud (1950), and Jacobs (1953).

This article summarizes the results of an extensive series of experiments which were made to test a number of arthropods (17 species) as possible vectors of *Toxoplasma gondii*.

As donors in attempts to infect arthropods, rabbits, guinea pigs, chicks, and pigeons were used. These animals were infected by intraperitoneal or intradermal inoculation with the RH strain of *Toxoplasma*. This strain of the parasite was isolated by Sabin (1941) from a fatal human case and has since been carried in mice; it is highly virulent for all of the animals used, except chicks. Assurance that the donors actually carried the infection is given by the fact that all rabbits and guinea pigs died within the expected period following inoculation; acute infections in rabbits have been demonstrated always to result in a high parasitemia. The same has been found true of acute infections in birds (Jacobs and Jones, 1950). The chicks and pigeons that did not die were shown to have an infection by inoculation of their blood into mice.

Rabbits, guinea pigs, hamsters, rats, mice, voles, chicks, and pigeons were used as recipients to which transmission was attempted. With the exception of rats and chicks, all are known to be highly susceptible to the RH strain of *Toxoplasma*. Tests for the presence of *Toxoplasma* in these hosts were done, after suitable incubation periods, as follows: The animals were dissected and portions of the liver, spleen, and brain were preserved in Orth's fixative for future sectioning if necessary.

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[1] The writers are indebted to Charles B. Evans, Paul C. Shade, Edith Edwards, Solomon S. Green, Jr., Henry A. Highland, and Mary Adams Heron for technical assistance in carrying out the details of the experiments on which this report is based.

Impression smears from these organs were examined following fixation in methyl alcohol and staining with Giemsa. Other portions of the same organs were ground together in saline and inoculated intraperitoneally into 2 mice. At the end of 1 week, 1 of these mice was killed and smears of its tissues examined. After 1 or 2 more weeks, the second mouse was killed and smears of its tissues prepared, and 2 more mice were subinoculated from it. These latter animals were then killed and examined at 1 and 2 weeks. In the later experiments some subinoculations were continued in mice through as many as 6 blind passages; these will be described in the appropriate sections under "Results." The method was the same as that employed by Jacobs, Melton, and Jones (1952) in a survey of wild pigeons. In some cases, before sacrificing, blood was taken from the birds and inoculated into mice as a test for its infectivity.

In some experiments, arthropods were tested by direct inoculation into mice to see if they had acquired parasites during their feeding. Suspensions of the crushed bodies or expressed gut-contents of the arthropods were prepared in saline and each lot so treated was inoculated intraperitoneally into 2 mice, which were then examined and subinoculated as described above. Also, in a few cases, the feces of arthropods were collected and either inoculated into mice or fed to the animals.

Arthropods were exposed to infection by feeding on 1 or more donor hosts, or on especially prepared infective materials. The arthropods were tested for the presence of *Toxoplasma* and were fed on susceptible hosts immediately after taking infectious meals and at varying times thereafter. This provided for testing the possibilities of simple survival of the toxoplasmas within the arthropod, and for incubation and possible transformation of the parasite. Arthropods were held at around 30 degrees C. during the off-host periods.

RESULTS

The essential data have been summarized in Tables 1 and 2. Entirely negative results were obtained with the 9 species of arthropods listed in Table 1. Occasional positive results were obtained with the 8 species listed in Table 2, but no particular species was found to give consistent evidence that it might serve as a vector of toxoplasmosis. As determined by direct tests, done by inoculation into mice of the macerated tissues and gut contents of the arthropods, toxoplasmas remained viable and infectious within the bodies of all the species named in Table 2, except *Amblyomma americanum*. There is no way to ascertain the percentage of infected individuals in a particular lot of arthropods thus shown to be positive for toxoplasmas, but the inconsistent results of various tests on different lots of the same arthropod species would suggest that the number of infected individuals was low. In many of the cases, the arthropods were tested before the blood-meal had been digested, and the positive finding may have been due merely to the survival of the toxoplasmas within this favorable milieu. The survival of parasites beyond the period of digestion of the blood is consequently of more significance. The positive results will be evaluated in detail below.

Rhodnius prolixus, Triatoma phyllosoma pallidipennis, and *T. rubrofasciata.* Survival of toxoplasmas within the gut of these bugs was demonstrated in 8 out of 29 lots, and in *R. prolixus* and *T. rubrofasciata* the parasites remained viable for at least 6 days. In all cases, undigested blood was present in the gut; in no instance

were the bugs found to retain toxoplasma after the complete digestion of the blood-meal.

Rhipicephalus sanguineus. Only 1 positive result was obtained in direct tests of 40 lots of ticks. The one lot of 45 unengorged adult ticks which proved positive was tested by direct inoculation into mice 30 days after the nymphs had completed engorgement on a recipient rabbit and 60 days after the larvae had fed on an inoculated rabbit. All 3 stages of the two previous generations had fed on inoculated rabbits. The macerated bodies of this lot of ticks were inoculated into 2 mice. These mice appeared perfectly normal, and after 2 and 3 weeks they were sacrificed and inoculated into another pair of mice. One of these second passage mice was found, by paracentesis, to have peritoneal exudate containing toxoplasmas. The parasites were of low virulence and required many passages in mice before they were

TABLE 1.—*Summary table: Arthropods tested as possible vectors of* Toxoplasma gondii *with negative results*

Species	Total numbers and stages fed on recipients after having fed on inoculated hosts	Tests for the presence of *Toxoplasma gondii*	
		Mammalian recipients	Arthropods tested by inoculation into mice
		Numbers	Numbers
Bdellonyssus bacoti (Hirst) Tropical rat mite	Several hundred thousand, all stages	96	Few hundred, all stages
Dermanyssus gallinae (DeGeer) Common chicken mite	. . . . .	. .	300, all stages
Psoroptes equi cuniculi (Delafond) Rabbit ear mite	Approx. 2000, all stages	2	700, all stages
Cimex lectularius Linnaeus Bedbug	Several thousand, all stages	41	534 nymphs and adults (7 lots)
Triatoma infestans (Klug) Conenose bug	105 nymphs and adults	1	105 nymphs and adults
Culex quinquefasciatus Say Southern house mosquito	2,544 adult females	12	194 adult females (16 lots)
	59 adult females (fed on infected peritoneal fluid)	. .	59 adult females (4 lots)
	12 adult females (fed on infected chick embryos)	. .	12 adult females (4 lots)
Pseudolynchia canariensis (Macquart) Pigeon fly	78 adults	7	1 adult
Xenopsylla cheopis (Rothschild) Oriental rat flea	Several thousand adults	57	396 adults (2 lots)
Ctenocephalides felis (Bouché) Domestic cat flea	Several thousand adults	35	406 adults (4 lots) Flea feces (3 lots)

capable of being maintained routinely. The history of this strain will be described by Jacobs and Melton in a separate report.

The rabbit recipient mentioned above was sacrificed 30 days after the nymphs had fed, and its tissues were inoculated into mice. Six blind passages were made in mice, but no *Toxoplasma* infection was demonstrated.

Thus the infection may have been acquired by the larval *Rhipicephalus* while feeding on the infected host and retained for 60 days through the later development stages of the tick. There is also the possibility that the infection may have been acquired by the ticks during feeding on infected hosts in either of the two previous generations and been transmitted through the eggs. The great difference in virulence, as compared with the original RH strain, of the strain recovered from the mice injected with tick material makes the positive result difficult to evaluate. It is believed unlikely that the toxoplasmas were derived from a latent murine infection

TABLE 2.—*Summary table: Arthropods tested as possible vectors of* Toxoplasma gondii *with positive results*

Species and purpose	Total numbers and stages fed on recipients after having fed on inoculated hosts	Tests for the presence of *Toxoplasma gondii*			
		Mammalian recipients		Arthropods tested by inoculation into mice	
		Numbers	+ cases	Numbers	+ cases and survival time
Rhodnius prolixus Stal Conenose bug	23 nymphs	1	0	20 (5 lots)	1 lot of 4. Few minutes 1 lot of 4. 6 or more days
Triatoma phyllosoma pallidipennis (Stal) Conenose bug	Near 1000 nymphs and adults	3	0	1206 (18 lots)	2 lots of 4 each. Under 4 hours 1 lot of 6. Over 20 hours
				1147 fed to 15 mice	0
Triatoma rubrofasciata (DeGeer) Conenose bug	43 nymphs	2	1?	46 (6 lots)	1 lot of 12. Few minutes 1 lot of 16 and 1 lot of 3. 6 or more days
Rhipicephalus sanguineus (Latreille) Brown dog tick					
Stage to stage transmission	193 nymphs and adults	7	0	1067 larvae, nymphs and adults (12 lots)	1 lot of 45 adults. 60 days
Transovarian transmission	Over 15000 larvae, 1309 nymphs and adults	55	0	Several thousand eggs and larvae	0
				890 larvae, nymphs and adults (28 lots)	0
Pediculus humanus corporis DeGeer Human body louse	Several thousand, all stages	8	0	5130 (23 lots) Louse feces (1 lot)	1 lot of 200. Under 24 hours 2 lots of 100 each. 5 and 7 days or more
	171 nymphs and adults (fed on infected peritoneal fluid)	2	1		
Dermacentor variabilis (Say) American dog tick					
Acquisition and retention	.....	..	..	319 larvae, nymphs and adults (12 lots)	1 lot of 50 larvae. Over 24 hours
Stage to stage	788 larvae, nymphs and adults	32	1	327 nymphs and adults (11 lots)	0
Transovarian	4511 larvae, nymphs and adults	22	0	763 nymphs and adults (10 lots)	0
Dermacentor andersoni Stiles Rocky Mountain wood tick					
Acquisition and retention	.....	..	..	1020 larvae, nymphs and adults (19 lots)	2 lots of 3 adult females each. Under 24 hours
				Tick feces (7 lots)	1 lot of 58 nymphs. Under 4 days
Stage to stage	619 nymphs and adults	15	0	92 nymphs and adults (7 lots)	0
Transovarian	7867 larvae, nymphs and adults	58	2	Approx. 6000 eggs	0
				2292 larvae, nymphs and adults (11 lots)	1 lot (part) of 83 adults
				Tick feces (2 lots)	0
Amblyomma americanum (Linnaeus) Lone star tick					
Acquisition and retention	.....	..	..	545 larvae, nymphs and adults (12 lots)	0
				Tick feces (2 lots)	0
Stage to stage	724 nymphs and adults	25	3	177 nymphs and adults (6 lots)	0
Transovarian	13 larvae	2	0	25000 eggs, 1000 larvae, 1 nymph (9 lots)	0

during the passages in mice. This belief is based on the general failure to find toxoplasmas in laboratory bred mice (Jacobs, Melton, and Jones, 1952).

Pediculus humanus corporis. The one rabbit which gave a positive test had been exposed for 19 days to 71 lice which had fed once upon infected peritoneal fluid 24 hours before being placed on the host. The peritoneal fluid was obtained from infected mice. Since the toxoplasmas were highly concentrated, the fluid was diluted to 1/5 to 1/18 with heparinized blood of rabbit. The insects were fed through chick-skin membrane. The numerous offspring of the original 71 lice also fed upon the host. The rabbit was sacrificed 34 days after the lice were removed from it. Toxoplasmas were demonstrated in the peritoneal fluid of mice of the fifth passage following inoculation of brain, liver, and spleen from the rabbit into two mice.

The second rabbit, which was not found positive, had been exposed to the bites of 100 lice which had fed once upon infected peritoneal fluid 24 hours before being placed on the host. Fifty of these lice had been placed on the host 6 weeks before sacrifice, while the remaining 50 lice had been placed on it 4 weeks before the sacrifice. The numerous offspring of the original 100 lice also fed upon the host.

Twenty-three lots, composed of 5,130 lice in all stages of development, were tested by the direct method for the presence of toxoplasmas. The lice had fed on inoculated rabbits during the last 6 or 8 days of infection. One lot of louse feces was also collected and similarly tested.

Toxoplasmas were demonstrated in 3 lots of lice by the following procedures. One of the 3 lots, composed of about 200 insects, was ground in saline and force-fed to mice within 24 hours after removal from the infected rabbits. Force-feeding was accomplished by introducing the suspension directly into the stomach by means of a hypodermic syringe fitted with a needle, the point of which was rounded with solder. The 2 other lots, each composed of 100 lice, were macerated in saline and inoculated intraperitoneally into mice 5 and 7 days respectively after removal from the inoculated rabbits. The mice in all these tests were shown to be infected by the microscopic demonstration of toxoplasmas in their tissues.

Survival of the *Toxoplasma* organisms in the lice may have been aided by the presence in the gut of undigested blood from either the infected or the uninfected hosts.

The lice of the latter 2 lots were fed during the 5 to 7 day interval between time of removal from inoculated hosts and time of the tests, on uninfected rabbits. Smears and subinoculations of the tissues of these rabbits into mice failed to reveal the presence of toxoplasmas.

Four other uninfected rabbits which served as hosts for these lice were tested by the subinoculation of their tissues into mice through 6 blind passages. In no case were they demonstrated to be infected with *Toxoplasma.*

Dermacentor variabilis. Of 23 lots of various stages of these ticks, tested by direct inoculation of their macerated bodies and gut contents into mice, only 1 was found positive for toxoplasmas. This lot comprised larvae which had completed engorgement from 1 to 5 days previously on an infected rabbit. Of 2 mice inoculated from them, 1 died of toxoplasmosis after 10 days; the other was found negative on sacrifice at 2 weeks. The extended survival time of the mice, and the fact that only 1 was infected would indicate either poor survival of the parasites within the

tick larvae or the acquisition of only a few parasites by the arthropods during engorgement.

One positive result was also obtained in tests of the transmission of *Toxoplasma* by the bite of these ticks. This was one of 17 rabbits which had been fed upon by adult ticks after previous feedings of the arthropods, as larvae and nymphs, on infected rabbits. In this instance 15 adult female ticks were used. The rabbit was proven positive by the demonstration of toxoplasmas in the second passage mice subinoculated with its tissues. The serum of this rabbit was negative prior to exposure, and showed a dye test titer of 1 : 16 at the time of sacrifice, 6 weeks after exposure.

The various periods in this passage were as follows: 5 to 6 weeks after engorgement of the larvae, 9 to 10 weeks after engorgement of the nymphs.

The same group of 15 adult ticks which fed on the above-mentioned rabbit did not produce infection when macerated and injected into mice.

In further tests for the presence of *Toxoplasma* in ticks after engorgement on inoculated rabbits, 57 adult females were ground up in saline at various intervals after feeding and fed to 12 uninfected mice. No positives were found among these mice or among others subinoculated from them.

Dermacentor andersoni. The presence of viable and infectious toxoplasmas was detected in 2 rabbits which had served as recipients in experiments to determine possible transovarian transmission. The larvae, nymphs, and adults of one generation of the ticks had been fed exclusively on inoculated rabbits. The 3 stages of the succeeding generation were fed exclusively on recipients. The 2 positive recipients had been fed upon to engorgement by 101 and 128 nymphs, respectively, of that succeeding generation. Periods which may have served for incubation of the toxoplasmas were approximately 4 to 8 weeks in the fed larvae and unfed nymphs, 5 to 10 weeks in the fed nymphs and unfed adults, and about 1 week in the fed adults of the first generation; 7 weeks in the eggs and unfed larvae, and 7 weeks in the fed larvae and unfed nymphs of the second generation. The period allowed for incubation in the mammalian recipients was 5 weeks.

The 2 positive rabbits mentioned above were among 18 upon which 1,465 nymphs of the above mentioned succeeding generation had fed. Periods allowed in the succeeding generation lay between 6 and 11 weeks in the eggs and unfed larvae, and between 3 and 10 weeks in the fed larvae and unfed nymphs. Three to 8 weeks were allowed for incubation in the mammalian hosts.

The presence of toxoplasmas in the 18 recipient hosts was sought by inoculation of tissues from the sacrificed recipients into mice, and subsequent subinoculation through 6 blind passages. Toxoplasmas were demonstrated in the peritoneal fluid and on Giemsa-stained smears of the tissues of sixth-passage mice inoculated originally from the tissues of the above-mentioned 2 rabbits. The serum of the 2 positive rabbits, taken before and after exposure, was negative on the dye test.

Thirty-seven lots of arthropods in various developmental stages were tested directly, by the inoculation of their macerated bodies into mice, to detect the presence of *Toxoplasma*. Also, 9 accumulations of the feces of such arthropods were tested. Positive results were obtained on 4 lots of arthropods. In 2 of these, each consisting of 3 adult females, the ticks had completed engorgement on infected hosts within 24 hours before they were tested. Toxoplasmas were demonstrated in peri-

toneal fluid and/or Giemsa-stained tissue smears of mice inoculated with their macerated bodies and gut contents, or in second-passage mice subinoculated from them. One lot of 58 nymphs was found positive in the same manner when tested about 4 days after engorgement on an inoculated host. They had fed as larvae on an inoculated rabbit 13 weeks earlier.

Only 1 case of retention of infectious organisms was found in which the last blood-meal of the ticks had been completely digested. This was a case of apparent transovarian transmission. Part of 1 lot of 83 unengorged adult ticks which were derived from a previous generation fed on inoculated rabbits, but which had not in any stage fed on infected animals, were inoculated into mice in the manner described above. One of 2 second-passage mice subinoculated from the original mice which received this inoculum was found positive on Giemsa-stained smears and the other died and was too decomposed for examination when found.

The history of rearing and exposing the previous generation of ticks is the same as that reported above for the arthropods which produced infections in recipient rabbits. The ticks of this second generation had been held for 10 weeks after the nymphal feed, which was on an uninfected rabbit. The holding temperature was 30 degrees C except for a five-weeks period (fifth through ninth weeks) during which it was 15 degrees C. This period of 10 weeks may have served as an incubation period in addition to those periods given above for the one and the succeeding generation up to the nymphal feed.

Whatever the means, the *Toxoplasma* organisms in this case survived not only in the absence of an undigested blood-meal in the gut, but through the egg stage and through the larval and nymphal molts.

Amblyomma americanum. The 3 positive rabbits among the mammalian recipients had been fed upon by 29 adult female ticks which had fed as larvae and again as nymphs on inoculated rabbits. Periods which may have served for incubation of the toxoplasmas were approximately 5 to 15 weeks in the fed larvae and unfed nymphs, 7 to 10 weeks in the fed nymphs and unfed adults, and 2 to 9 weeks in the mammalian recipients.

These recipients were 3 of 10 rabbits which had been fed upon by 170 adult female ticks which had fed as larvae and again as nymphs on inoculated rabbits. Periods allowed for incubation in the larger group were as given above except for an extension to 18 weeks in the fed nymphs and unfed adults, and to 10 weeks in the mammalian recipients.

The three positive cases were found by Giemsa-stained smears of the brain (tissue) but not by animal inoculation. No preexposure serum tests had been made upon the recipient hosts.

DISCUSSION

These experiments have not resulted in the incrimination of any one of the arthropods tested as an effective vector of toxoplasmosis. However, in no instance did the tests include all possible avenues or conditions of arthropod transmission. Also, while the species tried are representative of a variety of groups of arthropoda, they do not by any means include more than a small number of the various species which might be suspected as playing the role in the epidemiology of toxoplasmosis.

Difficulties in the detection of toxoplasmas in the study material, requiring extensive use of blind passages in order to demonstrate parasites, raise the question as to whether or not the few positive results obtained were due to inapparent infections in the laboratory animals used. The discovery of toxoplasmas in mice of the sixth blind passage, for instance, must be related to the presence of parasites in the original animal under investigation, or the work is futile. Some of us (Jacobs, Melton, and Jones, 1952) have already discussed this point in regard to a survey of the prevalence of toxoplasmosis in pigeons. There is no reason to believe that laboratory-reared mice are carriers of *Toxoplasma.* The only recorded instance of the presence of *Toxoplasma* in laboratory mice is recorded by Mooser (1950). Sabin (personal communication) has stated that Mooser's mice were obtained from an animal breeder who had just introduced wild mice into his colony in order to invigorate his stock. Search for inapparent toxoplasmosis in hundreds of laboratory-bred mice has yielded negative results. Therefore it is believed that the positive results obtained in mice represent infection in the original study animals. As far as rabbits and other rodents are concerned, spontaneous infections are sometimes found in these animals. In order to rule out these cases, the serological test results have been recorded wherever pertinent.

One other point deserves mention in regard to the difficulty of demonstrating infections. This is the question of the adaptability of strains of *Toxoplasma* to different hosts, following passage outside the warm-blooded animal. It has been the experience of several investigations that differences in the virulence of strains are encountered among parasites isolated from various hosts. A number of strains isolated from animals in nature have been of low virulence and difficult to demonstrate on passage in mice. At this stage of our knowledge, there is no way of ascertaining what changes may occur in strains during the period of incubation, if any, within arthropods.

On the basis of the above considerations, therefore, the meager success encountered in these stuides, may be interpreted a little more favorably. A few cases of transmission from infected to uninfected laboratory animals by means of arthropods, and a few cases of prolonged retention of the organism in arthropods in a viable and infectious condition, appear to have been demonstrated. These cases suggest leads which may prove helpful in the guidance of future investigations. The retention of the infectious organism by an arthropod for a few hours to a few days after acquisition and during the digestion of the blood-meal, provides at least a limited opportunity for transmission, mechanical or cyclic. A case in point would be infection through ingestion of the arthropod by an animal, if oral infection can occur. The demonstrated ability of the tested species to retain infectious organisms for extended periods beyond digestion of the blood-meal, and to transmit in even a low percentage of cases in the laboratory, suggests that the species having this ability, although perhaps themselves ineffective as vectors, may prove to be marginal vectors within groups of species, some species or strains of which may be important vectors. Also suggested is the possibility that different environmental conditions might lead to longer survival and effective transmission.

The positive results obtained in these experiments with *Dermacentor variabilis, Dermacentor andersoni,* and *Amblyomma americanum* thus provide particularly good leads. The species of the genera which are represented are well distributed

and attack a wide variety of wild and domestic mammals and birds. All active stages of *Amblyomma americanum* attach to man. Survival and passage through the egg and from stage to stage as appears to have happened in the case of *Dermacentor andersoni,* if substantiated, is highly significant. Other genera of ticks and mites are worthy of trial. Considering the prevalence of *Toxoplasma* in wild birds, several species of bird mites such as *Liponyssus sylviarum* and *Liponyssus bursa* should be tested.

The positive results with *Pediculus humanus corporis* provide a good lead, there being many other species of *Anoplura* attacking known hosts of *Toxoplasma.*

On the negative side, the apparent freedom from involvement of *Bdellonyssus bacoti* and *Xenopsylla cheopis* is particularly noteworthy since they are widely distributed and common ectoparasites of known natural hosts of *Toxoplasma.* A similar but probably less noteworthy situation applies to *Cimex lectularius, Culex quinquefasciatus, Pseudolynchia canariensis,* and *Ctenocephalides felis.*

SUMMARY

An investigation of 17 species of bloodsucking arthropods as possible transmitters of toxoplasmosis was carried out through an extensive series of laboratory experiments. The arthropods were fed on inoculated hosts, often on successive hosts, during the development and at the height of the parasitemia. Laboratory rabbits, guinea pigs, chicks, and pigeons served as donor hosts in attempts to infect the arthropods. Lice and mosquitoes were also fed on infected peritoneal fluid, and mosquitoes were fed on chick embryos. Subsequently, some of the fed arthropods were tested for the presence of *Toxoplasma* by inoculation into mice or by being fed to mice. The larger group was fed on known susceptible hosts. Laboratory rabbits, guinea pigs, hamsters, rats, mice, chicks, and pigeons served as potential recipients to which transmission was attempted.

Periods varying from a few minutes in some cases to several weeks intervened between contacts with donor hosts and testing of the arthropods, or feeding on recipient hosts. Periods varying from several days to several weeks intervened between exposure of the recipient to bites and its sacrifice for test, to allow for incubation of the toxoplasmas. Feces of some of the arthropods were also tested.

Bdellonyssus bacoti (tropical rat mite), *Dermanyssus gallinae* (common chicken mite), *Psoroptes equi cuniculi* (rabbit ear mite), *Cimex lectularius* (bedbug), *Triatoma infestans* (conenose bug), *Culex quinquefasciatus* (southern house mosquito), *Pseudolynchia canariensis* (pigeon fly), *Xenopsylla cheopis* (oriental rat flea), and *Ctenocephalides felis* (domestic cat flea) did not appear to transmit the causative organism by bite to susceptible hosts, nor to retain the organism in an infectious condition.

The conenose bugs, *Rhodnius prolixus, Triatoma phyllosoma pallidipennis,* and *Triatoma rubrofasciata,* and the brown dog tick, *Rhipicephalus sanguineus,* appeared to retain the organisms in an infectious condition for various periods following the ingestion of blood-meals from infected hosts, but not to transmit by bite to susceptible hosts.

Three ticks and 1 louse have shown apparent transmission in the laboratory. *Dermacentor variabilis* (American dog tick), *Dermacentor andersoni* (Rocky Mountain wood tick), *Amblyomma americanum* (lone star tick), and *Pediculus*

humanus corporis (human body louse) appear to have maintained the toxoplasmas for extended periods after infection, as shown by inoculation into mice. In attempts at transmitting the infection to rabbits by the bite of these arthropods, a low percentage of positive results was obtained. Infection in the ticks, *Dermacentor variabilis* and *Amblyomma americanum,* seems to have been acquired in the larval or nymphal stages and to have been transmitted in the adult stage. Infection in the tick *Dermacentor andersoni* appears to have been carried through the eggs from infection as larvae, nymphs or adults to all 3 stages of the succeeding generation and from the nymphs to hosts through the bite. Infection in the louse was acquired from infected peritoneal fluid and transmitted by bite to a rabbit.

These results are regarded as further experimental evidence supporting the view that toxoplasmosis may be arthropod-borne. Ticks and sucking lice are regarded as particularly good leads for further investigation.

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EXPERIMENTAL INFECTION OF THE CHINCHILLA WITH *HYMENOLEPIS NANA* VAR. *FRATERNA*[1]

G. C. Shelton[2] and W. S. Bailey[3]

Only a few reports are available concerning cestode infections in the chinchilla. Bracken and Olsen (1950) reported finding 7 chinchillas infected with the larval stage of *Multiceps serialis*. Newberne and Burnett (1951) reported another case of coenurosis which was presumed to be *M. serialis*. They also found the cysticercus of *Taenia pisiformis* in the liver of a chinchilla. Olsen (1950) observed several cases of *Hymenolepis nana* infection in a herd in which some animals apparently died as the result of the infection.

RESULTS OF EXPERIMENTAL INFECTIONS

Approximately 1,900 eggs of *Hymenolepis nana* var. *fraterna* were given to each of 6 chinchillas.[4] Two additional animals were given an undetermined number of eggs. The results of observations on the prepatent and patent periods for the 8 chinchillas are summarized in Table 1.

Table 1.—*Prepatent and patent periods observed in chinchillas following experimental infections with* H. nana *var.* fraterna *eggs*

Animal no.	Sex	Prepatent period	Patent period
11-a	Female	15 days	Euthanasia
11-b	Male	15 days	Euthanasia
24940	Female	15 days	17 days
24813	Male	16 days	38 days
22327	Female	Animal never became infected	
P-7	Male	22 days	16 days
10903	Male	16 days	29 days—death of animal
20816	Female	18 days	51 days

Chinchillas 11a and 11b were sacrificed 16 days following their infection, and 51 and 44 worms respectively were recovered from the lower portion of the small intestine. This represented 2.68 and 2.32 per cent of the total eggs administered.

In an attempt to determine the effect on the chinchilla of repeated heavy infections of *Hymenolepis* eggs one animal (No. 20816) was given a mixture of feed and mouse feces for 11 consecutive days. Although this chinchilla developed an extremely heavy infection no symptoms suggestive of an intestinal disturbance were noted. The appetite was good throughout the infection. No appreciable change in body weight was noted, and the condition of the fur did not appear to be altered.

Chinchilla 10903 was used for studies on experimental giardiasis but because of the concurrent infection with *Hymenolepis* and the good example of an apparent internal autoinfection which developed, the case history of this animal will be considered. On April 19 an unknown number of *Hymenolepis* eggs were adminis-

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[4] The chinchillas used for this experiment were supplied by the Evergreen Chinchilla Ranch, Columbus, Georgia.

tered to this chinchilla with a dose of *Giardia* cysts. From May 4 through May 22 "a few eggs" were passed in the feces, indicating a rather mild infection. From May 22 until May 29 no eggs were demonstrated. Beginning May 29 and continuing until the death of the animal on June 4, extremely large numbers of eggs were discharged daily. The chinchilla was suffering from a severe *Giardia* infection with a watery diarrhea which first began May 19. Autopsy examination revealed *Hymenolepis* adults from the pyloric opening to the cecum with no special point of concentration. Counts made from aliquot samples of the total contents of the intestinal tract indicated that more than 14,000 adult worms were present.

The history of the infection in this animal leaves little doubt that the animal was suffering from internal autoinfection. Hunninen (1936) demonstrated that internal autoinfestion with *H. nana* can occur in the mouse when the resistance of the host becomes sufficiently low. This principle of internal autoinfection might explain the deaths reported by Olsen (1950).

SUMMARY

1. The prepatent period of *H. nana* in the chinchilla ranged from 15 to 22 days and the patent period from 16 to 51 days in the animals studied.

2. The percentage development of eggs to mature worms was 2.68 and 2.32 in two chinchillas examined. These worms were found well within the lower half of the small intestine.

3. An apparent instance of internal autoinfection in the chinchilla is described.

4. With the exception of Chinchilla 10903 (also infected with giardiasis) no clinical symptoms of disease were noted in any of the chinchillas.

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MEGALOCOTYLE TRITUBA N. SP. (TREMATODA: MONOGENEA)*

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Folda (1928) described *Megalocotyle marginata* from *Sebastodes nebulosus,* taken in Puget Sound, Washington, as a new genus and species. Both Price (1939) and Sproston (1946) not only accepted the genus as valid, but extended it to include two European species unlike the proposed species.

Eight individuals of the proposed species were taken from the gills of *Sebastodes paucispinus* (Ayres) collected offshore from Newport, Lincoln County, Oregon, February 24, 1951. Seven specimens were prepared as whole mounts, while the eighth one was serially sectioned. Subsequently 6 more specimens were taken from the same host and locality on July 21, 1952.

Megalocotyle trituba n. sp.

(Figs. 1–6)

Description: With the characteristics of the genus as described by Folda (1928) and amended by Price (1939). Body flattened dorso-ventrally and slightly convex on dorsum. Color alive and preserved, white. Length of type specimen 9.5 mm., breadth at anterior margin of testes 3.3 mm. of each. Anterior suckers (0.94×0.62 mm.) somewhat elliptical in shape, posterior margin with an indentation. Mouth located on mid-ventral line, near level of posterior edges of anterior suckers. Buccal cavity passes antero-dorsally and then posteriorly to end at region of cirrus duct (Fig. 1). There is no intestinal cecum.

Diameter of opisthaptor including membrane 2.75 mm.; without an expanded central part of the posterior area. No chitinized rods in muscular rim. Anterior hook 0.155 mm.; middle hook 0.110 mm.; posterior hook 0.130 mm.; this last "D"-shaped and not extending on either side beyond muscular rim of opisthaptor.

Cirrus duct, uterus and vagina 3 separate tubes from their respective points of origin to the genital atrium. Egg at widest diameter 0.15 mm. (Figs. 1 and 2).

Type material: The type specimen is deposited in the U. S. National Museum and has been catalogued as U. S. N. M. Helm. Coll. 37403. Two paratypes deposited in the same collections are numbered 37403. Other specimens are in the collection of the senior author.

Host: Sebastodes paucispinus (Ayres). Location: Attached to the gills.

The new species is readily separated from *Megalocotyle marginata.* The largest specimen recorder by Folda was 4.43 mm. long; the shortest of this form was 6.7 mm., the longest 9.5 mm. The breadth of the new species is at least twice as great as that of *M. marginata.* The sizes of anterior suckers in the two species likewise do not overlap, and shapes of suckers probably are significantly different. The posterior sucker or haptor is much larger in *M. trituba* and the outlines of the posterior septae are distinctly different. The posterior pair of hooks are formed differently in the two species. Chitinized rods are visible on the type specimen of *M. marginata,* made available by courtesy of the Director of the U. S. National Museum. No chitinized rods were present on the rim of the opisthaptor of *M. trituba.*

Folda pictured intestinal ceca with numerous diverticula extending the length of the body. The present writers were unable to see the ceca, even when using a phase microscope. However, on *Megalocotyle trituba* the reduced digestive tract was outlined with certainty by using serial sections along with a number of whole mount specimens. No intestinal ceca were present.

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The cirrus duct and uterus of *M. marginata* join to run as a common duct ending at the genital atrium. The new species name, *M. trituba,* was chosen to emphasize the fact that the three genital ducts all are separate until they reach the genital atrium.

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Explanation of Plate I

Fig. 1. Ventral view of the type specimen of *Megalocotyle trituba.*
Fig. 2. An enlarged drawing of the reproductive system.
Fig. 3. The middle hook from the opisthaptor.
Fig. 4. The posterior hook from the opisthaptor.
Fig. 5. The anterior hook of the opisthaptor.
Fig. 6. The opisthaptor, showing position of hooks and shape of posterior area.

PLATE I

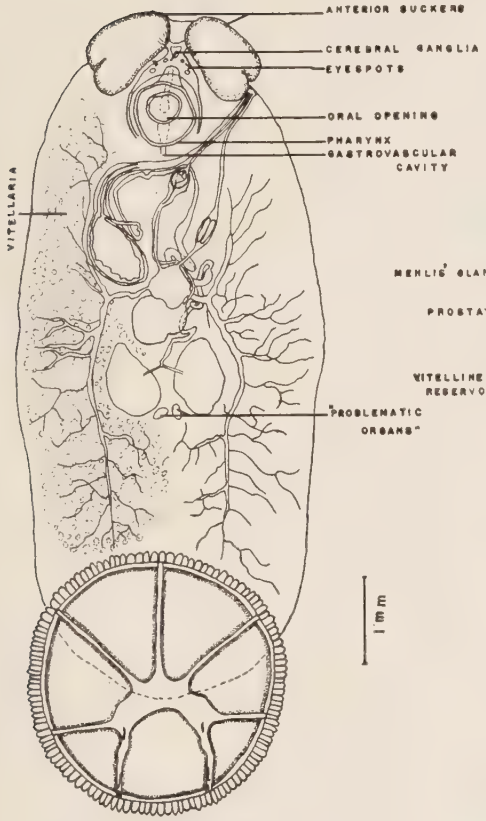

Fig. 1 Ventral view

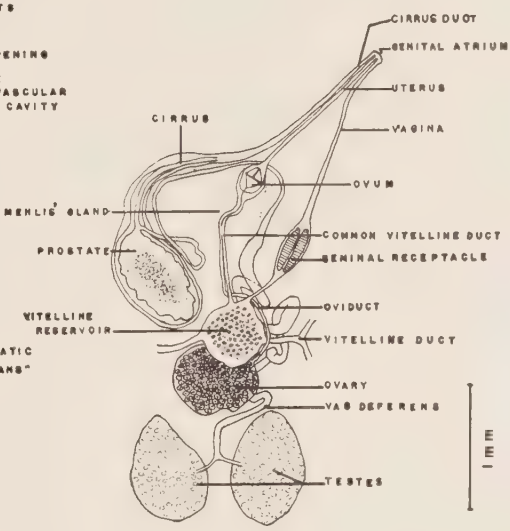

Fig. 2 Reproductive Organs
(Vitellaria omitted)

Fig. 3
Middle

Fig. 4
Posterior

Fig. 5
Anterior

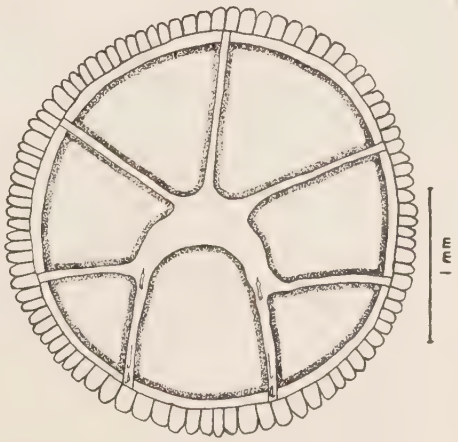

Fig. 6 Opisthaptor

STUDIES ON THE HELMINTH FAUNA OF ALASKA. XVI. A SURVEY OF THE HELMINTH PARASITES OF PTARMIGAN (*LAGOPUS* SPP.)

BERT B. BABERO[1]

References concerning the diseases and parasites of ptarmigan are widely dispersed in the literature, and for the most part are not readily accessible. It is the purpose of this paper to report the helminth parasites obtained from 191 willow ptarmigan, *Lagopus lagopus* Linn.; 45 rock ptarmigan, *L. mutus* (Mont.); and 56 white-tailed ptarmigan, *L. leucurus* (Rich.). These birds were collected over a period of four years, and were taken from widely separated areas in Alaska. (Fig. 1.)

MATERIAL AND METHODS

Although most of the birds were examined while fresh, the viscera of some specimens were preserved in 10% formalin solution in order that they might be shipped from the field to the laboratory. Near the end of this survey, blood films also were prepared from 42 birds (willow ptarmigan). These were stained with Wright's blood stain and examined for filariid larvae. All helminths other than microfilariae were fixed in alcohol-formalin-acetic acid solution (AFA). The two stains employed for cestodes and trematodes were Semichon's acid carmine and aqueous alum cochineal. Lacto-phenol was the clearing agent used for nematodes.

THE HELMINTH PARASITES

One hundred and nine ptarmigan, approximately 37 per cent of the total number examined, were infected with helminth parasites; these comprised five species of nematodes, two of trematodes and four of cestodes. The results of these findings are summarized in Table 1.

TABLE 1.—*Summary of helminth parasites obtained from 292 Alaskan ptarmigan* (Lagopus *spp.*), *1949–1953*

Helminth*	Species: No. of each			No. infected of total	Per cent infected of total
	Rock 45	Willow 191	White-tailed 56		
	No. infected				
Nematoda:					
A. compar	5	11	10	26	8.9
T. tenuis		6		6	2.1
Capillaria sp.	1			1	0.3
Trichostrongyle (unid.)	1			1	0.3
Trematoda:					
L. variae	1	4	3	8	2.7
B. fuscata	11	16	6	33	11.3
Cestoda:					
R. urogalli	2	4	3	9	3.1
D. proglottina	8	3	5	16	5.5
H. galli	6	2		8	2.7
R. nullicollis	1			1	0.3

* Does not include *M. lagopodis* (see text).

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NEMATODA

Ascaridia compar (Schrank, 1790)

Huus (1928) and Brinkman (1922) reported *A. compar* as a common parasite of the willow ptarmigan in Norway (the latter reporting it under the name of *Heterakis magnipapilla* v. Linstow, 1906). The nematode has been observed frequently in Alaskan ptarmigan (small intestine), and has been obtained from birds

FIG. 1. Localities from which ptarmigan have been examined. Solid symbols represent willow ptarmigan; hollow symbols represent rock ptarmigan; symbols with dot represent white-tailed ptarmigan.

collected over much of the Territory. Infections ranged from 1 to 16 worms per host.

Through the courtesy of Mr. Munthe-Kaas Lund, Bergen Museum, Oslo, Norway, specimens of *A. compar* from the Norwegian willow ptarmigan were made

available for comparison with the Alaskan worms. They differed essentially in the number of post-anal papillae on male specimens, the Alaskan form having six pairs of papillae rather than five as has been found in the Norwegian worm. This difference is not considered sufficient to justify separation of the two series of nematodes into two different species.

Trichostrongylus tenuis (Mehlis, 1846)

T. tenuis was reported by Shipley (1909a) as occurring in the English red grouse, *Lagopus scoticus* Lath. It has also been reported from domestic fowl. Cram and Wehr (1934) cited records of its distribution in Europe, Asia, Africa and North America.

T. tenuis has been obtained in Alaska from the ceca of willow ptarmigan, only. The birds were collected from Cold Bay on the Alaska Peninsula, and from the Talkeetna Mountains, near Anchorage. The present report constitutes the first record of the species from North American ptarmigan.

Capillaria sp.

A single specimen of a female capillarid was recovered from the proventriculus of a rock ptarmigan, collected 40 miles north of Fairbanks. Shipley (1909a, 1909b) and Brinkman (1922) reported the occurrence of *Trichosoma longicolle* (Mehlis, 1831) in the intestine of the red grouse and ptarmigan (*L. scoticus; L. mutus*), respectively. Read (1949), in his review of the capillarids of North American birds, pointed out that *T. longicolle* is a synonym of *C. caudinflata* (Molin, 1858). Employing Read's key to the species of *Capillaria* in birds, the specimen from rock ptarmigan could be designated *C. caudinflata*. However, from the material on hand it is impossible to establish a definite identification.

C. contorta (Creplin, 1839), a species not considered in Read's key, was reported as occurring in the crops of pinnated grouse, *Tympanuchus cupido* (Linn), and the sharp-tailed grouse, *Pedioecetes phasianellus* (Linn), by Morgan and Hamerstrom (1941).

Unidentified Trichostrongyle

Several trichostrongyle nematodes were recovered from the small intestine of a rock ptarmigan collected at Adak, in the Aleutian Islands. These specimens were turned over to Mr. Merle Kuns, Purdue University, for identification. Because of his being called into the armed forces, study of this material has not been completed. In a communication received from Mr. Kuns, he suggested the probability that these specimens may belong to an undescribed genus. It is hoped that the identification of these worms can be given in a later publication.

Microfilaria lagopodis (Sambon, 1907)

Although no attempt was made to search for blood parasites of ptarmigan until this survey was nearly completed, nineteen of forty-two willow ptarmigan examined were positive for filariid larvae.[2] From Anaktuvuk Pass (North-Central Alaska), eleven of twenty-five birds examined were positive for these larvae, and from the Arctic Village region (lat. 68°8′ N., 145°32′ W.), eight of seventeen birds were

[2] Six of the forty-two ptarmigan from which blood smears were made harbored trypanosomes, probably *Trypanosoma lagopodis* Haaland, 1928.

positive. Infections ranged from one to seventeen worms per low-power field (100×).

Filaria smithi, a blood nematode from the red grouse, was originally described by Sambon (1907, as cited by Haaland, 1928). Although she considered the original description inadequate, Haaland (1928) was of the opinion that blood nemanematodes which she obtained from Norwegian willow ptarmigan were probably the same as those described by Sambon. She pointed out, however, that the specific name used by Sambon was preoccupied by Cobbold's species from the elephant and proposed the name *F. lagopodis* for these microfilariae. Haaland also presented a more detailed description of this species based on her material from willow ptarmigan.

Brinkman (1949) considered microfilariae which he also obtained from willow ptarmigan to be the same as those of Haaland. He was of the opinion that the generic name, *Filaria* Mueller, 1787, could only be used for the adult worm. Since only the microfilaria was known in this case, he proposed that the name be changed to *Microfilaria lagopodis.*

In general, the morphology of the blood nematodes from Alaskan ptarmigan agrees with the description given by Brinkman of specimens which he believed to be the same as those of Haaland. However, there seems to be some question regarding the presence or absence of a red-staining central body. Brinkman, in comparing Haaland's description of her specimens with that of Sambon, stated, "Sambon's specimens seemed to be smaller, and he had not mentioned the characteristic red-stained zone (central body) which seemed to be so prominent in Haaland's specimens. We should not, however, pay too much attention to these differences, as length is a matter of the microfilaria's state of development, and the figure given by Sambon (1907) is certainly of a very young larva. The red central body is far from constant in my specimens, which are certainly identical with the Haaland species. In my opinion one can with almost certainty assume Sambon's and Haaland's specimens to be identical." The writer observed that when "old" blood was used for films the worms often would not stain completely, and in some instances not at all. This might be another reason why the red-stained zone was not reported in Sambon's description.

Filaria sp. has been recovered from the subcutaneous tissue over the pectoral muscles of seven Alaskan spruce grouse, *Canachites canadensis* (Linn.). The several blood films examined from this host species were negative. The helminth parasites of Alaskan grouse will be reported in a separate publication and it is anticipated that a more complete identification of this adult nematode can be given at that time.

TREMATODA

Leucochloridium variae McIntosh, 1932
(Fig. 1)

Among a series of trematodes of the genus *Leucochloridium* Carus, 1835, described by McIntosh (1932) were *L. pricei,* from an Alaskan spruce grouse [*Canachites canadensis* (Linn.)] and *L. variae* from a Michigan warbler [*Mniotilta varia* (Linn.)]. Kagan (1952) in his revision of LEUCOCHLORIDIINAE, synonymized *L. pricei* with *L. variae,* since his studies revealed that the characters upon which McIntosh based the differentiation were subject to considerable variation. This

species was also reported by Mueller (1941) from a gallinaceous bird [*Bonasa umbellus* (Linn.)] collected in New Hampshire.

Specimens of *L. variae* were recovered from members of each species of Alaskan ptarmigan (Table 1), and the number of parasites ranged from 1 to 214 per bird. Morphologically, these trematodes agree with the original description. The present report constitutes new host records for *L. variae*.

As pointed out by McIntosh (1932), since grouse [and ptarmigan] are nonmigratory birds, the immature stages of this trematode probably occur in a snail of the immediate habitat. Although terrestrial gastropods in Alaska are limited as to species and abundance, they are widely distributed. A locally collected spruce grouse which harbored *L. variae* also had several snails [*Euconulus fulvous alaskensis* (Pilsby.)] in its ventriculus. *Stagnicola yukonensis* Baker and *Succinea strigeata* Pfeiffer (identified by Dr. Harald Rehder, U. S. National Museum) were collected at Circle, Alaska. Kagan (1951), in his historical review, included several records of snails of the genus *Succinea* reported as harboring sporocysts of *Leucochloridium*.

Brachylaima fuscata (Rud. 1819)

(Fig. 2)

This trematode has been observed more frequently in Alaskan ptarmigan than has any other helminth parasite. Infections ranged from 1 to 265 specimens per bird. *B. fuscata* (= *Brachylaemus fuscatus*) has been found to be of wide distribution in Alaska, having been collected from various points within an area bounded on the north by Anaktuvuk Pass, on the west by Kotzebue, and on the south by the Anchorage area.

B. fuscata has been recorded previously from *Bonasa umbellus* Linn. and *Centrocercus urophasianus* (Bonaparte). Besides recovering this trematode from the three species of ptarmigan in Alaska, the writer has also collected it from the ruffed grouse, *B. umbellus;* the sharp-tailed grouse, *P. phasianellus* and spruce grouse, *C. canadensis*.

CESTODA

Haploparaxis galli Rausch, 1951

The original description of *H. galli* from the rock ptarmigan constitutes the first record of this genus from gallinaceous birds, insofar as the writer is aware. In the present study, this species has been collected six times from the rock ptarmigan and twice from the willow ptarmigan. Infections have consisted of one to four worms per bird. The localities from which this parasite was collected were Tulugak Lake (Brooks Range), Fairbanks, Nome and Kotzebue.

Davainea proglottina (Davaine, 1860)

(Fig. 3)

D. proglottina has been observed to be one of the more common parasites of Alaskan ptarmigan. This cestode was recovered from birds of three general localities as follows: Tulugak Lake, Fairbanks, and the Kenai Peninsula (near Hope).

The specimens from Alaskan ptarmigan resemble *D. tetroensis* Fuhrmann, 1919, but that species differs in having a double row of rostellar hooks and a larger number of testes (about thirty). Segment number is not always a reliable charac-

ter in distinguishing the two forms. Although *D. proglottina* has been reported from related birds, such as grouse and partridge, there appears to have been no previous record of its occurrence in ptarmigan.

Raillietina urogalli (Modeer, 1790)

This cestode is known to occur commonly in the small intestine of the willow ptarmigan and the red grouse. In Alaska, *R. urogalli* has been recovered nine times, with infections ranging from one to four worms per bird. The rock and white-tailed ptarmigan constitute new host records for the species. The localities in which the infected birds were collected are Anaktuvuk Pass, Anchorage, and Talkeetna Mountains (90 miles north of Anchorage), and Lake Schrader (Romanzof Mountains of northeastern Alaska).

Rhabdometra nullicollis Ransom, 1909
(Figs. 4–6)

A cestode belonging to the genus *Rhabdometra* Cholodkovsky, 1906, was recovered from the small intestine of a rock ptarmigan, collected during the summer of 1951 at Lake Schrader. A review of literature revealed that several species of this genus have been reported from gallinaceous birds: *R. nigropunctata* (Crety, 1890) was reported from a Siberian partridge, *Perdix perdix* (Linn.); *R. odiosa* (Leidy, 1887) was reported from the sharp-tailed grouse in North America; *R. nullicollis* Ransom, 1909, also from North America, has been reported from the pinnated, sharp-tailed, and sage grouse; *R. tomica* Cholodkovsky, 1906, was described from the black grouse, and again later reported from the willow ptarmigan in Russia; and *R. cylindrica* Beddard, 1914, was reported from North African grouse.

Except for a minor difference, testes number, the specimens from the rock ptarmigan fit the description of *R. nullicollis* as given by Boughton (1937). The vaginal bulb situated close to the genital sinus, present in *R. nullicollis,* was not observed with certainty in the proglottids of the Alaskan material studied. The occurrence of this cestode in rock ptarmigan constitutes a new host record.

DISCUSSION

For the most part, the helminth parasites of ptarmigan appear to be widely distributed geographically and are not limited to a given ptarmigan species. Van Cleave and Rausch (1951), in their study of the parasites of eider ducks, pointed out that when suitable intermediate hosts are present under ecologically similar conditions, the local bird populations are, through food habits, exposed to infection by identical species of parasites. The findings of the present study seem to support this statement. Few species of trematodes parasitize ptarmigan. *Prosthogonimus ovatus* (Rud., 1803), reported from *Lyrurus tetrix* (Linn.) by Fediushin (1949), is the only species recorded prior to this study insofar as the writer has been able to determine. This may be attributable to the arctic and subarctic conditions under which these birds live, and the restricted geographical distribution of suitable snail intermediate hosts in such regions. On the other hand, species of nematodes have been frequently reported from ptarmigan. *A. compar,* for instance, is apparently geographically unrestricted in its range. *R. urogalli,* likewise, is of wide occurrence.

Although in the present study, no recognized pathological effects could be attributed directly to helminth parasitism, several of the worms recovered from Alaskan ptarmigan have been previously incriminated as causes of "die-offs" among certain game birds. Perhaps the most significant of these is the cecal nematode *T. tenuis* and the cestode *R. urogalli.* The etiological significance of these parasites in the health of the English red grouse has been reported in detail by Shipley (1909a, 1909b) and Leslie and Shipley (1911–12). The part that helminthiasis may play in the reduction of ptarmigan numbers in Alaska is undetermined. The study of the helminth parasites of these hosts, particularly as they effect young birds, should be emphasized in relation to general faunal investigations in the Territory.

Acknowledgment

The writer is especially grateful to Dr. Robert Rausch and Mr. Everett L. Schiller, of this laboratory, for their identification of all cestode-material and assistance rendered throughout this study.

SUMMARY

A summary of the helminth parasites of Alaskan ptarmigan (genus: *Lagopus*) is presented. Of 292 ptarmigan examined, 109 were found to be infected with one or more of eleven species of worms. From the willow ptarmigan, two trematodes, *L. variae* and *B. fuscata,* and two cestodes, *H. galli* and *D. proglottina,* are reported for the first time. The rock ptarmigan constitutes a new host record for the cestode species *R. urogalli, D. proglottina,* and *R. nullicollis.* An unidentified trichostrongyle (possibly belonging to a new genus) and the trematodes *L. variae* and *B. fuscata* are also reported from this host for the first time. Apparently, the helminth parasites reported herein from the white-tailed ptarmigan are the first published records from this host.

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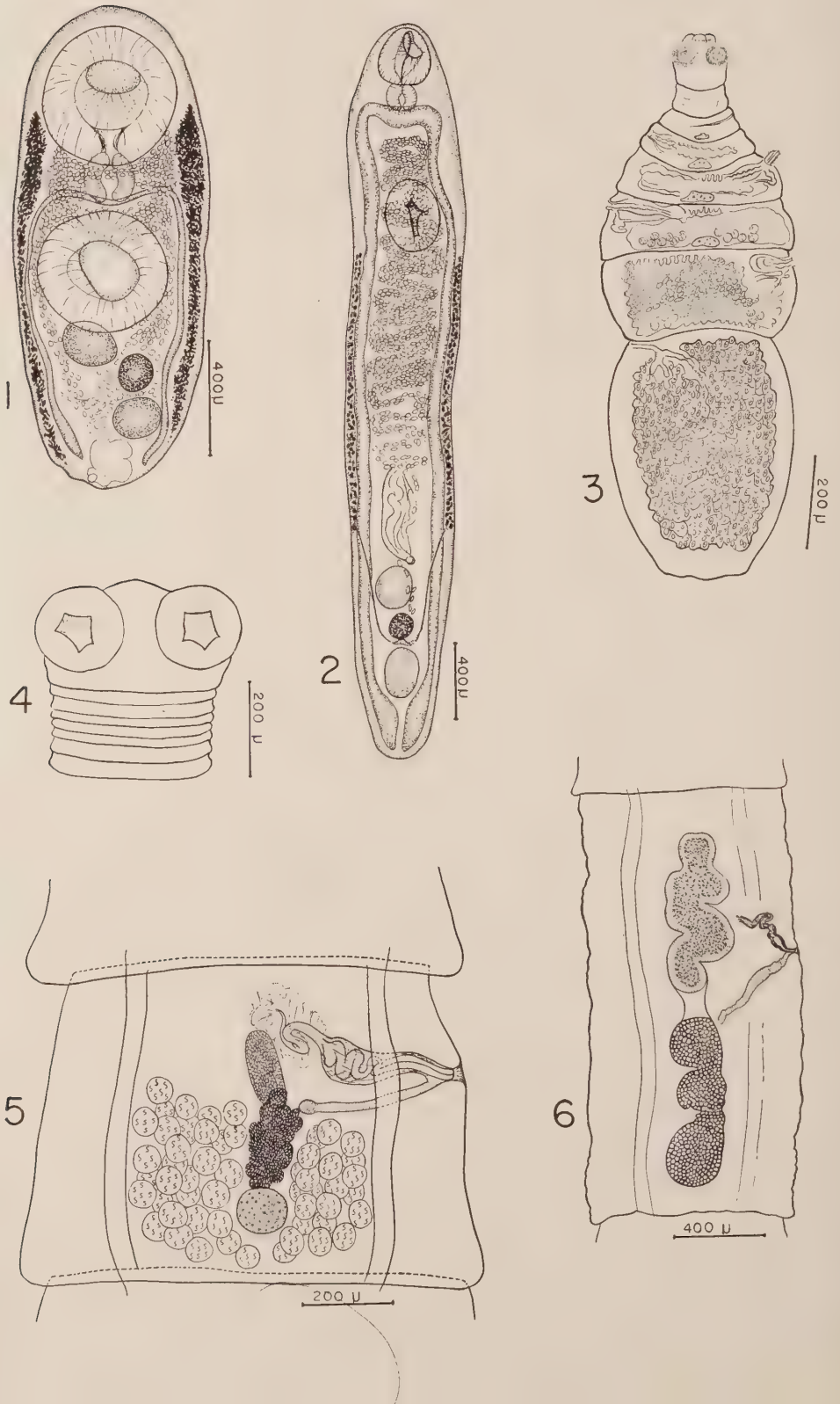
Plate I

Fig. 1. *L. variae*, ventral view.
Fig. 2. *B. fuscata*, ventral view.
Fig. 3. *D. proglottina.*
Fig. 4. *R. nullicollis*, scolex.
Fig. 5. *R. nullicollis*, mature proglottid.
Fig. 6. *R. nullicollis*, gravid proglottid.

PLATE I



THE EFFECT OF LOW TEMPERATURES UPON THE VIABILITY OF UNSPORULATED OÖCYSTS OF OVINE COCCIDIA[1]

EARL J. LANDERS[2]

The oöcysts of most species of *Eimeria* are resistant to the action of many chemical agents and to moderate fluctuations of temperature although they are susceptible to desiccation. It has been assumed by some investigators that low temperatures which occur in northern latitudes during the winter months are deleterious to the oöcysts although satisfactory evidence has not been presented. This paper presents the results of a laboratory investigation of the influence of low temperatures on the survival of unsporulated oöcysts of three species of ovine coccidia and the correlation of these results with available data on field temperatures at Laramie, Wyoming.

REVIEW OF LITERATURE

Very little has been done to determine the effect of low temperatures on ovine coccidia. Christensen (1939) investigated the effects of near-freezing temperatures on the viability of oöcysts of *Eimeria arloingi*. He demonstrated that at 0–5° C., the minimum time required for sporulation by oöcysts suspended in clear water is two weeks as compared with two to three days at room temperature. He further reported that when oöcysts were stored in a water suspension of fecal sediment at 0–5° C., one-fifth of the organisms survived for a period of 19 months and that none sporulated during the time of storage.

Pérard (1925) reported a 50 per cent or more kill at – 10 and – 15° C. of the oöcysts of two species of rabbit coccidia, *Eimeria perforans* and *E. stiedae*. However, exposure times were not given and the test used for viability was the appearance of the oöcysts.

The ability of oöcysts of avian coccidia to survive on soil under natural conditions has been determined by many authors, with several reporting survival for periods of over a year without loss of virulence. These experiments are considered incomplete in that adequate temperature records were not kept. Farr and Wehr (1949), in Maryland, reported survival of avian coccidia on soil in outdoor plots for 86 weeks and recorded minimum and maximum air temperatures during the course of the experiment.

MATERIALS AND METHODS

Four sheep, penned and maintained on a diet of native hay at the Wyoming State Veterinary Laboratory, Laramie, Wyoming, were used as a source of coccidia.

Hardcastle (1943) and Morgan and Hawkins (1948) recognized as valid nine species of *Eimeria* occurring in domestic sheep. Eight of these were encountered during the course of the study as well as *Eimeria honessi* Landers (1952). Since small numbers of oöcysts are produced by certain species, observations were limited to *Eimeria arloingi*, *E. nina-kohl-yakimovi*, and *E. parva*.

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Fecal samples were taken from the rectum and only well-formed pellets were used. Each sample was divided at random into a number of portions, one of which was used as a control while the others were used for exposure to low temperatures.

Recovery of the oöcysts from the samples was effected by a centrifugation-flotation technique. At least 100 oöcysts of each species were observed in each count. Each of the experiments was repeated at least once and the data presented are an average of all of the trials in a particular group.

Fecal pellets containing the oöcysts were placed in stoppered glass vials and exposed to the low temperatures. Two refrigerators were used. With one of these, a minimum temperature of – 19° C. was attainable. Temperature variation of this equipment was found to be ± 1° C. A cold box utilizing solid carbon dioxide as the refrigerant was constructed to obtain temperatures lower than – 19° C. This equipment was composed of two compartments so arranged that the temperature of one compartment could be regulated and maintained constant to ± 1.5° C.

Thawing was accomplished by placing the vials containing the frozen fecal material in the air at room temperature for one hour.

Attempts to condition the organisms to a low temperature of – 19° C. were made. Three temperatures other than –19 ° C. were used: 5, 0, and – 5° C. The samples were left for one hour at each temperature before moving them to the next lower temperature. This procedure was reversed for the warm-up process. When the organisms were conditioned prior to storage at – 25, – 30, – 40, and – 70° C., the samples were exposed to a temperature of – 19° C. for different periods of time after which they were moved to the lower temperature.

The effect of repeated freezing and thawing of oöcysts was tested by placing whole pellets in a stoppered vial and exposing the sample to the desired freezing temperature for a period of 6 to 24 hours. The samples were thawed by placing them in the air at room temperature for one hour. If the samples were to be refrozen, they were returned to the cold box at the previous low temperature for at least six hours.

Viability of the oöcysts before and after treatment was determined by observing the ability of the organisms to sporulate. Sporulation was accomplished by incubating the samples in a 2 per cent solution of potassium dichromate for four or five days at room temperature.

DATA

Low Temperature Survival

(1) *Oöcysts Exposed in Whole Pellets to – 40 and – 70° C.* Two procedures were followed in these experiments. In one series, samples were exposed directly to a temperature of – 40 or – 70° C.; in the other series, samples were conditioned at – 19° C. prior to storage at the lower temperatures. The conditioning periods ranged from four hours to seven days and oöcysts taken from these groups prior to storage at the lower temperatures always sporulated in numbers equal to the control. The minimum time the samples were exposed to the lower temperatures was two hours and the maximum, seven days. No viable organisms were recovered from any of the samples which had been exposed to either – 40 or – 70° C.

(2) *Oöcysts Exposed in Whole Pellets to – 30° C.* (Table 1). Samples were either exposed directly to – 30° C. or conditioned at – 19° C. for 24 hours prior to

TABLE 1.—*Viable organisms recovered from whole pellets stored continuously at -30° C.*

Conditions	Exposure time	% Sporulation		
		E. arl.	*E. nina.*	*E. parva*
Control	..	96.0	98.0	96.0
Frozen directly at – 30° C.	4 hours	50.0	49.0	48.0
	24 hours	0.0	0.0	0.0
Conditioned at – 19° C. for 24 hours prior to storage at – 30° C.	24 hours	96.0	97.0	95.0
	2 days	0.0	0.0	0.0
Conditioned at – 19° C. for 24 hours both prior to and after storage at – 30° C.	24 hours	95.0	94.0	96.0
	2 days	0.0	0.0	0.0

exposure. In one group, samples were conditioned at – 19° C. both prior to and after exposure.

(3) *Oöcysts Exposed in Whole Pellets to – 25° C.* (Table 2). Samples were either exposed directly or conditioned at – 19° C. for 24 hours prior to exposure. One series of tests was conducted in which the samples were conditioned at – 19° C. both prior to and after exposure.

TABLE 2.—*Viable organisms recovered from whole pellets stored continuously at – 25° C.*

Conditions	Exposure time	% Sporulation		
		E. arl.	*E. nina.*	*E. parva*
Control	..	94.0	96.0	92.0
Frozen directly at – 25° C.	1 day	94.0	95.0	92.0
	3 days	93.0	94.0	91.0
	7 days	91.0	92.0	90.0
	14 days	46.0	49.0	23.0
Conditioned at – 19° C. for 24 hours prior to storage	1 day	94.0	95.0	92.0
	3 days	92.0	93.0	90.0
	7 days	84.0	83.0	69.0
	14 days	43.0	45.0	19.0
Conditioned at – 19° C. for 24 hours both prior to and after storage	1 day	94.0	96.0	91.0
	3 days	91.0	90.0	89.0
	7 days	86.0	85.0	77.0
	14 days	43.0	41.0	18.0

(4) *Oöcysts Exposed in Whole Pellets to – 19° C.* Samples were exposed directly or conditioned at 5, 0, and – 5° C. both before and after exposure. No ill effects were noted after exposure for as long as 60 days.

(5) *Oöcysts in Whole Pellets Repeatedly Frozen at – 19, – 25, and – 30° C.* (Table 3). Samples were stored at the low temperature for at least six hours during each freezing period. Each time the samples were thawed, they were left at room temperature for one hour after which they were returned to the same low temperature for refreezing.

TABLE 3.—*Viable organisms recovered from whole pellets stored intermittently at low temperatures*

Freezing temperature	No. of times frozen	Exposure time total	% Sporulation		
			E. arl.	*E. nina.*	*E. parva*
– 19° C.	0	..	98.0	96.0	96.0
	7	96 hrs.	98.0	95.0	95.0
– 25° C.	0	..	95.0	94.0	88.0
	1	48 hrs.	92.0	94.0	87.0
	2	5 days	94.0	94.0	86.0
	3	6 days	92.0	93.0	85.0
	4	7 days	91.0	93.0	85.0
	5	8 days	91.0	89.0	83.0
	6	9 days	68.0	49.0	11.0
– 30° C.	0	..	89.0	85.0	89.0
	1	24 hrs.	0.0	0.0	0.0

Air and Ground Temperatures at Laramie, Wyoming

Deaton and Frost (1941) reported air and soil temperatures at Laramie, Wyoming for the years 1937, 1938, and 1939. Temperatures were recorded at one or two-hour intervals over each 24-hour period and the records for each such period then averaged. During the winter months for these years, the daily atmospheric temperature was invariably some 10 to 20° F. below the daily soil surface temperature.

FIG. 1.

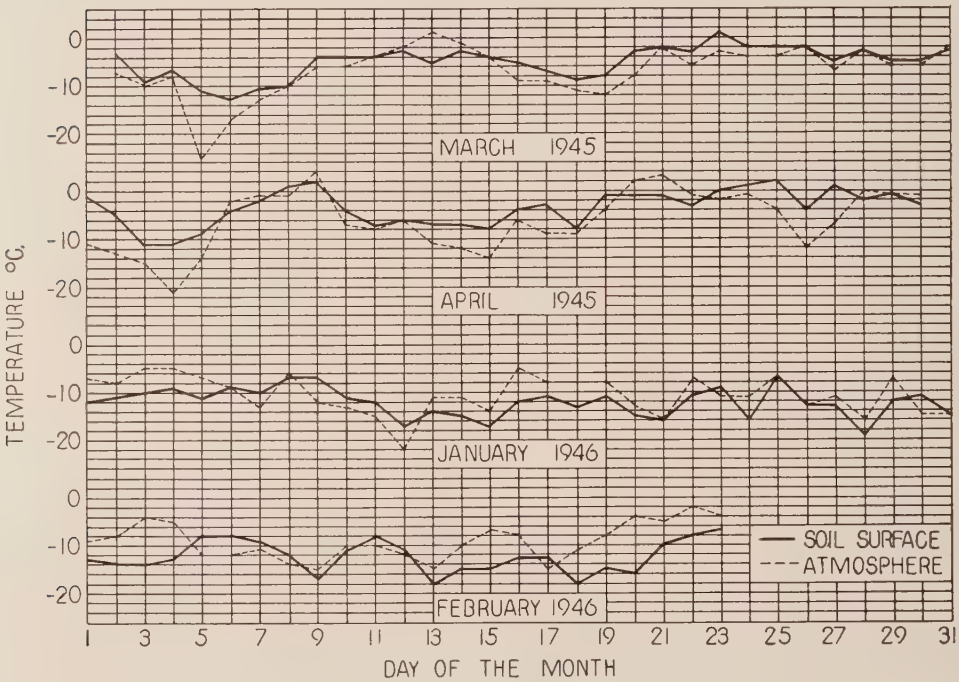

Figure 1 is a graph of the minimum daily air and ground surface temperatures at Laramie, Wyoming for March and April of 1945 and January and February of 1946. The soil surface temperature records are unpublished data which were recorded on the University of Wyoming campus by Dr. Robert Pfadt of the Department of Entomology and Parasitology. The atmospheric temperatures were recorded by personnel on the University of Wyoming campus and the records collected and published by the U. S. Department of Commerce, Weather Bureau (1945, 1946). The lowest soil surface temperature recorded during these months was – 19° C.

DISCUSSION

The oöcysts eliminated by sheep during the winter months may remain on the surface of the soil and thus be exposed to the temperatures which occur at this level. Since development of the oöcysts is inhibited at temperatures of 0–5° C., many may remain in the unsporulated stage for long periods in some areas in the northern latitudes.

The data on surface soil temperatures during the winter months at Laramie, Wyoming are available for only a limited number of years and while these records may not be typical of the most severe winters they are regarded as representing the usual conditions in this locality. From these data one could predict that the lowest soil surface temperature during an average winter would be between −15 and −20° C. During the years covered by the records, the minimum air temperature recorded was −25° C. while the lowest soil surface temperature was −19° C.

Figure 1 also indicates that the periods of lowest soil surface temperatures are of short duration, lasting less than 24 hours. The experiment in which unsporulated oöcysts were conditioned at −19° C. and then stored at −30° C. for 24 hours suggests that oöcysts could tolerate such field temperatures for 24 hours—which would exceed a field temperature period of −30° C. by several hours. Since the coldest periods of a winter are always preceded by periods of slightly higher temperatures, similar conditioning of the oöcysts under field conditions could be expected.

The evidence presented demonstrates that the unsporulated oöcysts of *Eimeria arloingi, E. nina-kohl-yakimovi,* and *E. parva* may undergo prolonged or intermittent exposure to a temperature of −19° C. in the laboratory without decrease in viability. It is probable that exposure to a like temperature under such natural conditions as would prevent the action of dessication would also prove innocuous.

The results of the experiments in which the unsporulated oöcysts were stored at −25° C. suggest that this temperature is very near the minimum survival temperature of the organisms or a little below it. Survival of the oöcysts stored at this temperature for periods of up to seven days was not appreciably altered, whereas exposure for 14 days did result in a reduction of viability of 50 per cent or more. A lower survival rate was found in this group in the oöcysts of *Eimeria parva* than in the oöcysts of the other two species. The results of intermittent storage of the oöcysts at −25° C. show that the organisms tolerate freezing at this temperature at least five times without significant decrease in viability.

The evidence indicates that the unsporulated oöcysts of the three species of *Eimeria* investigated will not survive prolonged exposure to temperatures of −30° C. or lower.

SUMMARY

Prolonged exposure to temperatures of −19 and −25° C. is innocuous for the unsporulated oöcysts of *Eimeria arloingi, E. nina-kohl-yakimovi,* and *E. parva* and when properly conditioned, they may be stored at −30° C. for 24 hours without decrease in viability.

A comparison is made of the air and ground surface temperatures at Laramie, Wyoming for five winters which reveals that the soil surface temperature rarely, if ever, reaches the extremes of low fluctuation which occur in the air masses. The available evidence indicates that the unsporulated oöcysts of these species of *Eimeria* will not normally be killed by the lower winter temperatures in this area.

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LEUCOCHLORIDIUM PERISORISAE, A NEW SPECIES OF TREMATODE (*LEUCOCHLORIDINAE*) FROM THE OREGON JAY, WITH A DISCUSSION OF THE APPLICATION OF HOST–PARASITE RELATIONSHIPS TO THE TAXONOMY OF THIS GROUP

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Twenty-seven fully mature specimens of what appears to be a new species of fluke have been recovered from the intestinal contents of an Oregon jay, *Perisoreus o. obscurus* Ridgway, collected near Oakville, Washington during March 1951.

The worms were flattened under slight cover-slip pressure, fixed with formalin-acetic-alcohol mixture, and whole mounts were prepared from specimens stained with Semichon's aceto-carmin. Measurements were made on all of the specimens collected and are given in millimeters with average values included in parentheses. The drawing was prepared with the aid of a camera lucida.

Leucochloridium perisorisae n. sp. (Fig. 1)

Description: *Brachylaimidae.* Body slightly more than twice as long as broad, 1.42–2.02(1.80) long by 0.61–0.80(0.73) broad. Cuticle armed with small spines in region of pharynx and oral sucker. Oral sucker subterminal in position, 0.32–0.39(0.36) long by 0.35–0.42(0.40) broad. Acetabulum equatorial in position, approximately round, 0.39–0.46(0.42) long by 0.40–0.48(0.44) broad. Pharynx attached directly to oral sucker without intervention of prepharynx, 0.11–0.14(0.12) long by 0.13–0.17(0.15) broad. Esophagus lacking. Intestinal crura extend from pharynx to posterior end of body, ending behind level of posterior testis and vitellaria. Genital organs typical of genus. Testes variable in size and shape, showing gradations from almost round, about size of ovary, to elongate ovoid, about twice as large as ovary. One testis frequently larger than other, although on average they are approximately same size. Testes situated in posterior part of body, with most anterior one on left (in two specimens the most anterior testis is on the right side of the body). In all 27 specimens anterior testis separated from acetabulum by distance greater than that separating posterior testis from end of body. Anterior testis 0.13–0.28(0.19) long by 0.09–0.20(0.13) broad. Posterior testis 0.12–0.28(0.19) long by 0.07–0.19(0.12) broad. Cirrus pouch 0.10–0.17(0.13) long by 0.05–0.08(0.06) broad. Cirrus unarmed. Ovary approximately round, 0.12–0.15 (0.13) long by 0.09–0.15 (0.13) broad, usually situated directly anterior to posterior testis with which it may be contiguous. In some specimens ovary is placed slightly medial to posterior testis, while in one specimen it is almost directly in line with both testes. Oviduct passes mesially from ovary to connect with common vitelline duct before turning anteriorly on right side as uterus (a fecundarium is present, but its connection with the oviduct was not observed). Uterus ascends from region of ovary through number of convolutions past right side of acetabulum into pre-acetabular region where it is always more or less extra-caecal near pharynx. Uterus then proceeds posteriorly on left side of acetabulum into region of genital organs where it opens through genital pore, situated at posterior tip of posterior testis, in region between ends of ceca. Eggs oval, 0.020–0.025(0.023) long by 0.011–0.013(0.012) broad. Vitellaria ventral (sometimes slightly but never completely lateral) to intestinal ceca and extend from level of pharynx to level of anterior tip of posterior testis. Collecting ducts pass mesially from posterior limits of vitellaria to unite near midline of body, forming common vitelline duct which may or many not be enlarged to form vitelline reservoir.

Host: Perisoreus o. obscurus Ridgway (Oregon Jay).

Habitat: Intestine.

Locality: Oakville, Washington.

Type: Slide No. 37199, bearing the type specimen has been deposited in the Helminthological Collection of the U. S. National Museum.

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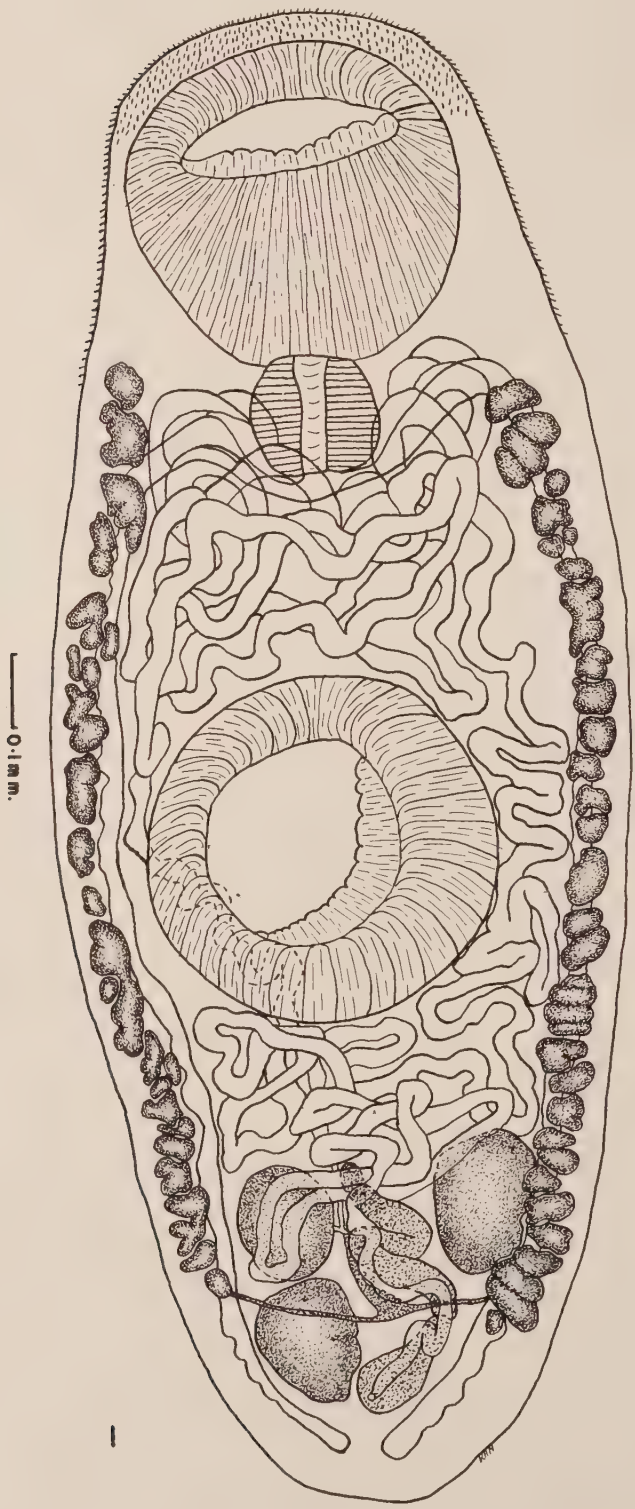

FIG. 1. *Leucochloridium perisorisae* n. sp., ventral view of mature specimen.

DISCUSSION

A recent revision of the subfamily *Leucochloridinae* Poche, 1907, by Kagan (1952) has greatly clarified the relationships of the various species previously assigned to the genus *Leucochloridium* Carus, 1835. Kagan retained ten species within this genus and transferred a greater number to the genera *Urogonimus* Monticelli, 1888, and *Neoleucochloridium* Kagan, 1952. However, as noted by Kagan, the problem of establishing valid criteria for the separation of the species of this subfamily is still essentially unsolved. Two characters which have been extensively employed to differentiate species of this group are distribution of vitellaria and arrangement and relative size of the gonads. According to McIntosh (1932) variation in these characters may occur among different individuals of a species because of lack of uniformity in preparation for study, but in a large, uniformly treated sample the individuals of a species are fairly uniform and similar. Hsu (1936) considered these criteria to be valid for species separation and added the distribution of the uterus and position and relative size of suckers. It is the author's impression that Robinson (1947) has very succinctly formulated the fundamental problem of helminth taxonomy in his statement, "the problem of *what constitutes a species* becomes of practical importance when one must determine the affinities of a trematode." Robinson believed that the foregoing characters and others have been relied upon to too great an extent in the classification of species of *Leucochloridium* since a number of the species of this genus can be separated on the basis of their descriptions only with great difficulty and little certainty. Robinson was uncertain of the validity of the color-pattern of the sporocysts as a species criterion. On the other hand, Kagan (1952) accepted this character and employed genital pore position, vitellaria distribution, and relative sucker size in a key to the species of *Leucochloridium*. Previously Kagan (1951) reported experimental results obtained by rearing specimens of *Leucochloridium cyanocittae* McIntosh and *Neoleucochloridium problematicum* (Magath, 1920) from metacercariae which led him to believe that the distribution of the vitellaria may not be a good specific character. However, it is the writer's impression that Kagan has drawn some conclusions from this data which may not be entirely realistic, and this appears to be particularly so in respect to the latter species. Kagan pointed out that the unnatural host for *N. problematicum*, the chick, yielded very poor results in infection experiments, while the natural hosts, gruiform birds, gave uniformly very good results. In regard to extent of vitellaria, Kagan reported that all specimens recovered from the unnatural host had short vitelline fields, while in those from the natural hosts the vitellaria always extended into the region of the cirrus pouch. On the basis of these observations Kagan concluded that the posterior extent of the vitellaria is under host influence and that therefore it is not grounds for the separation of species of *Neoleucochloridium*. It is the writer's belief that data obtained from helminths or other organisms which have been cultured under apparently abnormal conditions, i.e., in unnatural hosts, can not be utilized with any great assurance in establishing the normal morphological characteristics of a species. Kagan's data indicated that in addition to morphogenetic disturbance, an unnatural host may also quite drastically affect the viability of the helminth. In feeding experiments which included 14 gruiform birds (rails, coots, and gallinules), 17 chicks, and 25 other passerine and anserine birds, Kagan observed that only the

gruiform birds and five of the chicks were successfully infected with *N. problematicum*. It was noted that the former hosts harbored an usually large number of adults per host (12) while the five infected chicks harbored only ten individuals. The question arises as to whether or not the many species of this subfamily (described on the basis of a very few specimens, in some cases one or two individuals) aren't actually aberrant specimens of other species which have become distorted by an association with an unnatural host. On the basis of Kagan's data it appears likely that any naturally occurring, heavy infection probably indicates that the infected host is a natural one for the parasite, although the possibility remains that light infections may occur in natural hosts because of variations in viability. The writer considers the infection reported in this paper and which is comprised of 27 fully mature individuals to be a good representation of the variability found in the proposed new species, if not in the entire genus *Leucochloridium,* which species have developed under comparatively natural conditions. Actually the above considerations are similar to those originally offered by McIntosh (1932).

To facilitate the comparison of the distribution of vitellaria relative to the genital organs and intestinal crura and also the relative size and arrangement of the gonads, camera lucida drawings of the postacetabular region of all the present specimens were prepared. From these it was seen that the posterior extent of the vitellaria, the intestinal crura and their relations to other organs were quite constant. These observations are in agreement with those of Kagan (1951) on the 146 experimentally reared specimens of *Neoleucochloridium problematicum* from 14 gruiform birds, the natural hosts for that species. On the other hand, the relative size and arrangement of the genital organs of *Leucochloridium perisorisae* n. sp. were found to be highly variable. According to Kagan (1951) a similar situation obtains for specimens of *Leucochloridium cyanocittae* McIntosh reared in canaries, sparrows, a wren and a starling, all of which are non-corvid birds. Kagan noted, in contrast to the present observations, that in his experimental specimens of *L. cyanocittae* that the posterior extent of the vitellaria was also quite variable. It should be noted that the viability of metacercariae of *L. cyanocittae* in Kagan's experiments was usually quite low, and furthermore, Kagan apparently performed no experiments to determine the comparative normality of his experimentally reared specimens. One wonders whether or not they were fertile. As previously pointed out, it would appear that in order for data from experimental specimens to be taxonomically valid, the organism should be cultured under conditions which are, at least, nearly optimum for the growth and development of the species and which are similar to those obtaining in infections in natural hosts. It appears that until more extensive and satisfactory evidence is available that the distribution of the vitellaria in an extended series of uniformly prepared specimens is satisfactory in conjunction with other characters (i.e., relative sucker size, extent of intestinal crura, color-pattern of broodsac, position of genital pore) for species differentiation. While there is little doubt that experimental methods are sorely needed in helminth taxonomy, it seems quite apparent that their application and interpretation of results should be approached with great caution. The primary task of a taxonomist is to identify organisms recovered from wild populations and therefore, it appears unsound to base such a taxonomy on abnormal individuals reared under laboratory conditions which for various ecological reasons may not be available in the natural environment.

Apparently in all the recognized species of *Leucochloridium* the posterior extent of the vitellaria and intestinal crura are very nearly the same. In contrast to this situation, in *Leucochloridium perisorisae* n. sp. the intestinal crura extend well past the posterior limits of the vitellaria in all of the 27 specimens examined. The new species may be separated from the more closely related species of the genus as follows:

Leucochloridium melospizae McIntosh, 1932, and *L. paradoxum* Carus, 1835, both have oral suckers which are larger than the acetabulum, while in *L. perisorisae* n. sp. the situation is reversed.

Leucochloridium passeri Wu, 1938, has a nonspinose integument while in the new species there is a marked spination in the region of the oral sucker.

Leucochloridium australiense Johnston and Simpson, 1940, has a markedly different distribution of its vitellaria. In this species the vitellaria are extra-caecal and they extend from the middle of the oral sucker to the posterior limits of the posterior testis. In *L. perisorisae* n. sp. the vitellaria invariably overlap the intestinal crura (in a few specimens the overlapping is not complete and there is slight lateral displacement) and they begin at the level of the pharynx and end before reaching the caudal boundary of the posterior testis.

SUMMARY

A new species of *Leucochloridium* Carus, 1835, is described from the Oregon jay, *Perisoreus o. obscurus* Ridgway. A discussion of some of the characters for species separation and of the application of life history studies to the taxonomy of this group is presented. The relationhips of *Leucochloridium perisorisae* n. sp. to other members of the genus are discussed.

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DIPLOPHALLUS ANDINUS N. SP. AND *MONOECOCESTUS RHEIPHILUS* N. SP., AVIAN CESTODES FROM THE HIGH ANDES

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A small collection of avian cestodes, taken in 1952 from two areas in the Peruvian and one locality in the Chilean Andes, was made available for study. The collection contained two whole cestodes and additional strobilar fragments from two avocets, a single cestode from a tinamou, and two whole and several incomplete cestodes from rheas; thirty-six non-gravid cestodes were available from one of the latter hosts taken in the Chilean locality.

The cestodes were fixed in Bouin's fluid and stained with Grenacher's borax carmine or Ehrlich's haematoxylin. The latter stain was used full strength or in highly dilute aqueous solution. Inasmuch as the specimens were extremely thick, the cuticular and muscular layers were scraped off in an attempt to reveal the underlying structures. This method did not yield satisfactory results. Optimum results were obtained when thick frontal and transverse sections were cut by hand from unstained specimens and subsequently stained with dilute Ehrlich's haematoxylin. Thin sections were cut with the aid of a microtome and stained with haematoxylin and eosin.

Unless otherwise stated, all measurements are given in millimeters.

We wish to thank Dr. and Mrs. Oliver P. Pearson and Dr. Carl B. Koford, Museum of Vertebrate Zoology, University of California, Berkeley, for collecting and making available these interesting cestodes.

Diplophallus andinus n. sp.

Diagnosis: Diplophallus: Strobila about 6 cm. long by 7.0 and 7.5 in greatest width. Proglottids much wider than long. Scolex with four suckers and well developed rostellum without hooks; scolex diameter 0.672 and 0.722; sucker diameter range 0.252–0.269; rostellum length 0.248 and 0.264, rostellar sac width 0.142. Ventral, longitudinal excretory ducts with ramified cross-connections in each proglottid. Longitudinal musculature in two adjacent, prominent layers. Testis number 59–88, average 73 per segment; testis diameter range 49–69 μ, average 62 μ; cirrus pouch length 0.247–0.363, average 0.313 in mature proglottids, up to 0.520 long in gravid proglottids; cirrus pouch width 0.115–0.148, average 0.135 in mature proglottids, up to 0.172 wide in gravid proglottids. Cirrus with internal, longitudinal rows of small spines, and with spiny, cone-shaped, readily detached cap. Uterus tubular, later sacciform. Eggs with three membranes, 33–36 × 43–50 μ; onchosphere 20 μ in diameter.

Type host: *Recurvirostra andina* Philippi and Landbeck.

Habitat: Small intestine.

Type locality: Pampa Huaitire, 14,500 feet, Tutupaca, Department of Moquegua, Peru.

Type: U.S. Natl. Mus., Helm. Coll. No. 48724.

The scolex (Pl. I, Fig. b) bears four muscular suckers and a well developed rostellum. Though Cohn (1900) described the rostellar armature of *Diplophallus polymorphus* as consisting of ten strong hooks, no trace of hooks was observed in our specimens. Posterior to the short neck the strobila gradually broadens and thickens with the maximum width being attained in the semi-gravid region. Pro-

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glottids are typically wide and short. The internal organs are first observed a few millimeters posterior to the scolex. The gravid proglottids are well demarked, with adjacent segments connected by a thin and transparent membrane, apparently cuticular. Eventually, segments may become completely separated except for small areas in the region of the longitudinal excretory duct. The slightest tension on such a chain will cause separation of segments. The gravid region is the thickest portion of the strobila; muscular contraction may result in intersegmental separation of the muscles in this region. Generally the cirri are extruded (particularly so in the gravid region) and may be easily observed with the unaided eye.

Cohn (1900) reported a poorly developed longitudinal musculature for *D. polymorphus*. In our species the longitudinal musculature consists of two prominent layers (Pl. I, Fig. e). Each muscle is composed of bundles, the number of which decreases in the lateral regions of the segment where the muscles are smaller. The muscles are most prominent near the middle of a segment. The transverse musculature is also well developed, particularly adjacent and peripheral to the longitudinal layer. Transverse fibers are seen between and central to the two longitudinal layers. Numerous dorso-ventral fibers are present. In addition, there is a weakly-developed cortical longitudinal musculature.

The excretory system consists of two pairs of longitudinal ducts, a broad ventral pair and another much narrower medial and dorsal pair. The ventral ducts have a variable number of cross-connections in the posterior half of each segment. The transverse cross-connections are extensively branched (Pl. I, Fig. a) and form an irregular network of supplementary ducts of variable width and extent. Contrary to the statement of Cohn (1900) regarding *D. polymorphus,* no ramifications of the narrower, dorsal ducts were observed in the present species. In some proglottids the ramifications of the ventral ducts were observed to extend toward the region of the female gonads, but this seemed to be exceptional.

Male reproductive system: There are two complete sets of male reproductive organs, each consisting of a group of testes, a vas deferens and a muscular cirrus pouch. The latter organ contains a small seminal vesicle and a partly extruded cirrus. The testes are small, spherical bodies and lie lateral to the ovary in the anterior half of the segment. They lie in several dorso-ventral planes extending laterally to the dorsal excretory ducts and medially a little beyond the lateral limits of the ovary (Pl. I, Fig. a). The vasa efferentia were not observed. The vas deferens is a narrow and thin-walled tube which passes dorsal to both excretory ducts to lead into the pear-shaped muscular cirrus pouch. The cirrus is peculiar in several respects. It is quite long, the extruded portion measuring up to 0.709. The extruded cirrus is sufficiently long to reach and enter an adjacent posterior segment, and sufficiently strong to penetrate the cuticular and muscular tissues of this adjacent segment. Such penetration was observed in several instances. Since a functional vagina is not present, fertilization appears to occur by the cirrus penetrating the cuticle and entering the parenchyma. Cohn (1900) disagreed with Wolffhügel (1900) who claimed that in the absence of a functional vagina, the cirrus must penetrate the parenchyma. Wolffhügel actually observed cirri imbedded in the parenchyma. Cohn argued that such penetration would depend on the presence of a special structure on the cirrus. A weak and narrow cirrus, as

seen in *D. polymorphus,* could not possibly effect penetration. He therefore claimed that Wolffhügel's conclusion must have been based on an artifact.

In the present material certain structures were found which may clarify this question of cirrus penetration. The cirrus (Pl. I, Fig. d) is heavily armed with numerous spines. There are several rows of small spines arranged longitudinally within and extending to the tip of the cirrus. In addition, the tip of the cirrus bears a spinous cap (Pl. I, Fig. c). The numerous spines of the cap are oriented in different directions. Since both cirrus and cirrus pouch are muscular, it is likely that penetration is effected by a combined action of spines and muscles. As the cirrus is withdrawn from the parenchyma, the cap probably remains inside the proglottid. The unattached cap seems to be a very fragile structure; several fragmented caps were found on our slides. The cap was not often seen, the majority of the cirri having lost them. Fragmented caps were not found within the parenchyma but this is not surprising since the spines are very fine and the segments are very thick and opaque.

Female reproductive system: The single female gonad occupies the greater portion of the segment between the longitudinal excretory ducts; it measures 1.68 to 1.76 in width in proglottids 3.02 to 3.86 wide (Pl. I, Fig. a). Numerous delicate, finger-like projections extend anteriorly from the ovarian margin. The vitelline gland is slightly lobed and lies immediately behind the ovary. The two uterine tubes, narrow and thin-walled in early mature segments, extend laterally to the longitudinal excretory ducts. More posteriorly in the mature region, small outpocketings begin to develop at the tips of the uterine tubes, becoming more extensive as development proceeds. Gradually, the uterus becomes sac-like and in gravid segments occupies most of the space between the longitudinal muscles (Pl. I, Fig. e).

Plate I, figure f illustrates egg structure. It is not certain whether or not the eggs found in our specimens are fully mature. Many of the terminal proglottids did not contain eggs. Three membranes surround the onchosphere. The outer membrane is thin and delicate and, in most eggs examined, this membrane had at least one kink or fold. The intermediate membrane is as thin as the outer one and invariably bears at its two poles a more or less spherical, cell-like structure. The innermost membrane is relatively thick. In some eggs two slender filaments connect the innermost and intermediate membranes. The inner membrane is usually thickened at its two poles. The onchosphere is nearly spherical and has six slender hooks.

It has been mentioned above that there is no functional vagina in our specimens. However, Cohn (1900) and Wolffhügel (1900) described as vaginae in *D. polymorphus* two blindly ending sacs, ventral to the ovary and anterior to the uterus. We found similar structures in our specimens (Pl. I, Fig. a). These are thin-walled and finely granular in appearance and upon close examination are seen to contain delicate thread-like structures which may be spermatozoa. It seems probable that these sacs function as seminal receptacles, but their origin from patent vaginae is questionable. The presence of a "shell" gland could not be established with certainty.

The detailed and careful description of *Diplophallus polymorphus* (Rudolphi, 1819) by Wolffhügel (1900) and additional information available from Cohn (1900) make it possible to compare *D. polymorphus* with *D. andinus* in some detail. Both

authors described an armed rostellum for *D. polymorphus* while our species lacks hooks. The longitudinal muscles in *D. polymorphus,* although similar to those in *D. andinus* in type and distribution, are smaller and very much elongate in cross-section. In *D. andinus* the longitudinal muscles are almost circular in cross-section. In his drawings of the muscular system, Wolffhügel (1900) did not show transverse fibers internal to the longitudinal muscles and stated that such fibers are rarely found. On the other hand, in *D. andinus* there are transverse muscle layers internal and external to the longitudinal muscles, as well as between the longitudinal layers.

There are definite differences in cirrus armature in the two species of *Diplophallus.* In *D. polymorphus* the cirrus is covered with spines arranged in steep spirals (Wolffhügel, 1900). In *D. andinus* the spines do not cover the cirrus but are restricted to several longitudinal rows inside the cirrus and to the spiny cirrus cap. A spiny cap has not been described for *D. polymorphus,* but it is possible that both Wolffhügel and Cohn simply failed to find it since this is a very fragile structure.

Egg structure is similar in the two species of *Diplophallus* in that there are three membranes surrounding the onchosphere, the innermost membrane being thickened at its two poles. There is a filamentous connection between the innermost and the middle membranes in the eggs of both species. However, in *D. polymorphus,* these filaments originate at the same pole of the innermost membrane and terminate close to each other on the middle membrane (Wolffhügel, l.c.), whereas in *D. andinus* similar filaments project laterally, but terminate at opposite positions on the middle membrane. The polar, cell-like structures on the middle membrane are apparently absent in *D. polymorphus.* In *D. polymorphus,* the length-width ratio of the egg is much greater than in *D. andinus.*

Certain other specific differences in the morphology of the two species may be summarized as follows: Scolex diameter in *D. polymorphus* is 1.6 and in *D. andinus* up to 0.722; the testis diameter is $36 \times 29\ \mu$ in *D. polymorphus* and $62\ \mu$ (average) in *D. andinus;* the cirrus pouch is 0.250×0.250 in *D. polymorphus* as compared to 0.313×0.135 in *D. andinus;* the eggs of *D. polymorphus* are $91 \times 46\ \mu$ and in *D. andinus* the eggs are about $46 \times 35\ \mu$. The above measurements of *D. polymorphus* are Wolffhügel's. Cohn (1900) obtained somewhat different data from his specimens: the scolex diameter was 0.440, the testis diameter $60 \times 40\ \mu$, and the cirrus pouch 0.140×0.172. While such discrepancies in measurements may be due to variability of the species, it is also possible that Cohn and Wolffhügel were dealing with different species of *Diplophallus* from *Recurvirostra avocetta.* With regard to structural detail, Cohn and Wolffhügel did not differ significantly in their descriptions. Cohn did not mention spines on the cirrus of his specimens although he reported a smaller number and size of rostellar hooks.

Thus, the species described herein differs from *D. polymorphus* in the musculature, the cirrus armature and egg structure. There are also differences in the relative size of some of the organs, but they are difficult to evaluate in view of the lack of agreement in measurements reported for *D. polymorphus* by previous workers.

THE TAXONOMIC POSITION OF DIPLOPHALLUS AND SOME RELATED GENERA

The genus *Diplophallus* was erected by Fuhrmann (1900) for the reception of *Taenia polymorpha* Rudolphi. This species was studied in some detail by Wolff-

hügel (1898; 1900) and Cohn (1900). Fuhrmann (1907) and Ransom (1909) referred *Diplophallus* to the family ACOLEIDAE. The last named author included in this family the genera *Acoleus, Dioicocestus, Gyrocoelia,* and *Shipleya.* Fuhrmann (1909, 1911) added the genera *Progynotaenia* and *Proterogynotaenia* to the family. Meggitt (1924) referred the genera *Monoecocestus* Beddard, *Urocystidium* Beddard, and *Diploposthe* Jacobi to ACOLEIDAE. In his comprehensive study of avian cestodes, Fuhrmann (1932) pointed out that since *Monoecocestus* is an anoplocephalid and *Urocystidium* is probably a larval taeniid, these genera cannot be retained in the ACOLEIDAE. In the same work Fuhrmann disagreed with Poche (1926) who had erected a family, DIPLOPOSTHIDAE, for the genus *Diploposthe,* a genus regarded by Fuhrmann as belonging to the HYMENOLEPIDIDAE. *Leptotaenia* Cohn was referred by Fuhrmann (1932) to the ACOLEIDAE.

The genera *Progynotaenia* and *Proterogynotaenia,* along with *Gynandrotaenia,* were subsequently referred by Fuhrmann (1936) to a new family, PROGYNOTAENIIDAE. Southwell (1930) placed *Dioicocestus* in a separate family, DIOICOCESTIDAE, because these forms are dioecious. Burt (1939) accepted the concept that the dioecious forms merit family rank and added to the family *Infula* Burt, *Shipleya* Fuhrmann, and *Gyrocoelia* Fuhrmann; like *Dioicocestus, Infula* has separately sexed individuals, and Burt has argued quite persuasively that *Shipleya* and *Gyrocoelia* probably have a similar separation of the sexes. More recently, Wardle and McLeod (1952) have assigned the above mentioned genera and others to the families ACOLEIDAE, containing only the genus *Acoleus;* DIOICOCESTIDAE, containing the genera *Dioicocestus, Infula, Shipleya* and *Gyrocoelia;* and DIPLOPOSTHIDAE containing the genera *Diploposthe, Diplophallus, Diplogynia,* and *Jardugia.* The last named workers did not refer *Progynotaenia, Proterogynotaenia,* or *Leptotaenia* to families, considering them to be of uncertain taxonomic position.

Fuhrmann's insistence upon retaining *Diplophallus* in the family ACOLEIDAE merits consideration. *Diplophallus* closely resembles *Acoleus* not only in lacking a vagina but in the nature of the musculature. In examining sections of *Diplophallus* the pattern of development of the longitudinal and transverse muscles is a striking feature. The major difference between *Acoleus* and *Diplophallus* seems to reside in a single feature, the duplication of the male reproductive organs in each segment in *Diplophallus.* Three of the four species of *Acoleus* and both species of *Diplophallus* are known only from charadriform hosts. The significance of the doubling of the male genitalia in *Diplophallus* seems comparable to the significance of duplication of the genitalia occurring in genera of other cestode families such as the ANOPLOCEPHALIDAE and DILEPIDIDAE. In the opinion of the present authors *Acoleus* and *Diplophallus* should be retained in the same family.

Monoecocestus rheiphilus n. sp.

Diagnosis: Strobila about 12 cm. in total length, by 5.0 to 6.0 in maximum width. Scolex unarmed, 0.823–1.075 in diameter; suckers range from 0.319–0.336 by 0.386–0.453 in diameter (average 0.361 × 0.425). Excretory system consists of two pairs of longitudinal ducts, the median pair of which is connected by a transverse duct in posterior part of each proglottid, and with longitudinal anastomosis between transverse connections. Genital pores alternate regularly or irregularly. Testes from 24–55 in number, in continuous field between longitudinal excretory ducts, posterior and lateral to ovary; they are spherical in shape and range 43–50 μ (averaging 46 μ) in diameter. Cirrus sac 0.330–0.561 (average 0.422) in length; cirrus sac 0.122–0.171 wide (average 0.140). Cirrus covered with numerous small spines. Vagina opens into genital atrium anterior to cirrus sac. Ovary slightly displaced toward poral side. Uterus

sac-like in gravid proglottids. Eggs spherical, 60–72 μ in diameter, average 64 μ; onchosphere with well developed pyriform apparatus and measures 17 μ in diameter; with the pyriform apparatus, the onchosphere measures 23–26 μ in length.

Type host: *Pterocnemia pennata* (d'Orbigny).

Habitat: Intestine.

Localities: Pampa de Capazo, 14,300 feet, 120 km. S. Ilave, Department of Puno, Peru (Type) and Cerro Tatio, 14,000 feet, 50 mi. E. Calama, Prov. Antofagasta, Chile.

Type: U.S. Nat. Mus. Helm. Coll. No. 48722.

The unarmed scolex bears four muscular suckers situated on short, thick stalks (Pl. II, Fig. c). When contracted, these stalks form marked folds around the suckers. The scolex is followed by a narrow neck region of variable length, and, as segmentation begins, the strobila gradually broadens. The relaxed mature trapezoidal proglottids are craspedote. Semigravid and gravid proglottids are 1.17 and 1.51 thick and therefore difficult to study in stained whole mounds.

The musculature consists of well developed longitudinal and transverse layers. The longitudinal layer (Pl. II, Fig. b) is denser internally where the muscles consist of several bundles. Toward the periphery the muscles become smaller, consisting of a few or only one muscular bundle and are irregularly distributed throughout the parenchyma. The transverse layers are found on either side of and adjacent to the longitudinal muscles. The internal transverse layer is about twice as wide as the peripheral one. Dorso-ventral fibers were not observed.

The excretory system (Pl. II, Fig. a) is composed of two pairs of longitudinal ducts and of ramifications of the more medial longitudinal pair. The lateral longitudinal ducts are very narrow and adjacent to the medial ducts which are about five times as wide as the lateral ones. The transverse connecting ducts are large, being of about the same diameter as the large medial longitudinal ones. In addition to these, a prominent dorsal longitudinal anastomosis connects the transverse ducts near the midline; this central duct may have no further anastomoses, or, if situated in the aporal half of the proglottid, it usually has a relatively small transverse branch to the longitudinal duct on the aporal side. In some specimens, the medial longitudinal connecting anastomosis is of the same width as the large lateral longitudinal ducts.

In many specimens the genital pores alternate regularly, but occasionally two or three consecutive proglottids may have the pores situated on the same side. In a few specimens, alternation is quite irregular.

Male reproductive system: The spherical testes occupy a roughly U-shaped field situated behind and on both sides of the ovary, extending anteriorly to the anterior margin of the ovary on the aporal side and not quite this far on the poral side (Pl. II, Fig. a). The vas deferens is thick-walled and twisted, and, before entering the cirrus pouch, it enlarges considerably. As it enters the pouch, it leads into a compact and darkly staining seminal vesicle. The cirrus pouch is an elongate and muscular organ with a thick wall and contains a very much twisted cirrus. The cirrus is covered with numerous small spines. The genital atrium is well defined and communicates with the exterior by a narrow duct. In some proglottids, particularly contracted ones, the genital pore region appears as a prominent bulge on the lateral edge of the segment. The vasa efferentia were not observed.

Female reproductive system: The ovary, situated near the center of the proglottid slightly toward the poral side, has many digitiform lobes and partially

envelops the lobed vitelline gland. A small "shell" gland is present. From the region of the "shell" gland the vagina arises as a narrow, delicate tube. A short distance anterior to the origin of the vagina there is a spherical seminal receptacle, which stains very heavily when filled with sperm. The vagina continues anteriad and porad as a much enlarged, thin-walled tube, crosses the cirrus sac in the region of the median longitudinal excretory duct, and continues along the anterior side of the cirrus sac to enter the genital atrium.

In semi-gravid and gravid proglottids, the uterus is sac-like in outline and is filled with eggs. No trace of a uterus could be observed in the mature proglottid.

The eggs are roughly spherical; both the outer and inner membranes are thin and smooth. The onchosphere has a well-developed pyriform apparatus (Pl. II, Fig. d).

Monoecocestus rheiphilus resembles *M. anoplocephaloides* (Douthitt, 1915) in the position of the ovary and of the testes which are posterior and on either side of the ovary in both species. *M. anoplocephaloides* from the gopher, *Geomys bursarius,* is a much smaller form and differs markedly from *M. rheiphilus* in relative size of organs. *M. americanus* (Stiles) shares with *M. rheiphilus* the almost central position of the ovary which is more poral in *M. variabilis* (Douthitt).

DISCUSSION OF *Monoecocestus* BEDDARD

A single cestode belonging to this genus was collected from *Tinamotis pentlandi* Vigors, 5 km. E. Lago Suche, 14,600 feet, Department of Moquegua, Peru. Because of the dearth of material, specific identification of this form is deferred until such time as additional specimens are available. Our observations indicate that the *Monoecocestus* from tinamous is not cospecific with *M. rheipilus,* described above.

Prior to the present report the species of the genus *Monoecocestus* were known from mammalian hosts whose major common feature is the herbivorous habit. The recent life history studies of Freeman (1952) on the species from North American porcupines and of Melvin (1952) on the species from cotton rats indicate that, as with other anoplocephaline cestodes, the intermediate hosts of *Monoecocestus* species are free-living oribatid mites. This feature of the life cycle probably has importance in ecologically determining the characteristic distribution of the anoplocephalines among herbivorous animals.

The present finding of *Monoecocestus* in birds is of interest because of the extension of knowledge of host distribution. It takes on added interest with the realization that the avian hosts of *Monoecocestus* reported herein are grazing birds. They are large, essentially flightless birds living on the high, almost treeless Andean plateau. From the standpoint of the ecologist they probably occupy niches more like those of herbivorous mammals than of birds in general. This furnishes another example of the quandary of the parasitologist in evaluating the role of ecological and physiological factors determining host specificity.

SUMMARY

Two new species of cestodes are described from Peruvian birds. *Diplophallus andinus* n. sp. is described from the avocet *Recurvirostra andina,* and *Monoecocestus rheiphilus* n. sp. from the rheiform bird *Pterocnemia pennata. Monoeco-*

cestus sp. is reported from *Tinamotis pentlandi*. The taxonomic position of the genus *Diplophallus* is discussed.

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Explanation of Plates

Unless otherwise stated, all drawings are made with the aid of a camera lucida.

Abbreviations

c: cirrus
cm: cortical musculature
cp: cirrus pouch
ded: dorsal excretory duct
dvm: dorso-ventral musculature
lm: longitudinal musculature
o: ovary
sr: seminal receptacle
t: testes
ted: transverse excretory ducts
tm, tm1, tm2: transverse musculature
u: uterus
v: vitelline gland
vd: vas deferens
ved: ventral excretory duct

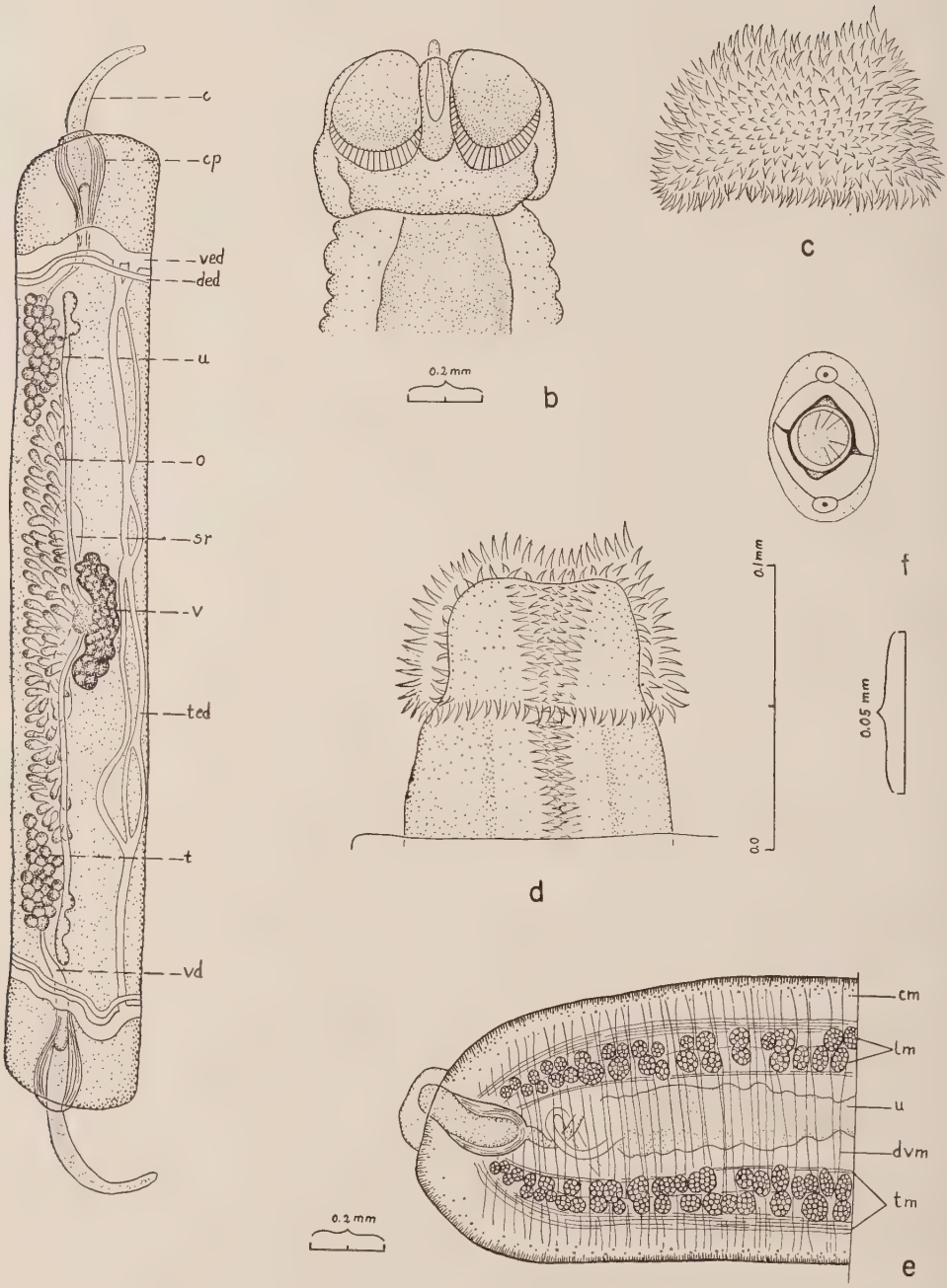

PLATE I

Diplophallus andinus

FIGS. a, Free-hand diagram of mature proglottid; b, Scolex; c, Cirrus cap; d, Optical section of tip of cirrus with spiny cap and longitudinal rows of spines; e, Part of cross-section through semi-gravid segment, showing musculature, uterus and cirrus pouch; f, Embryonated egg.

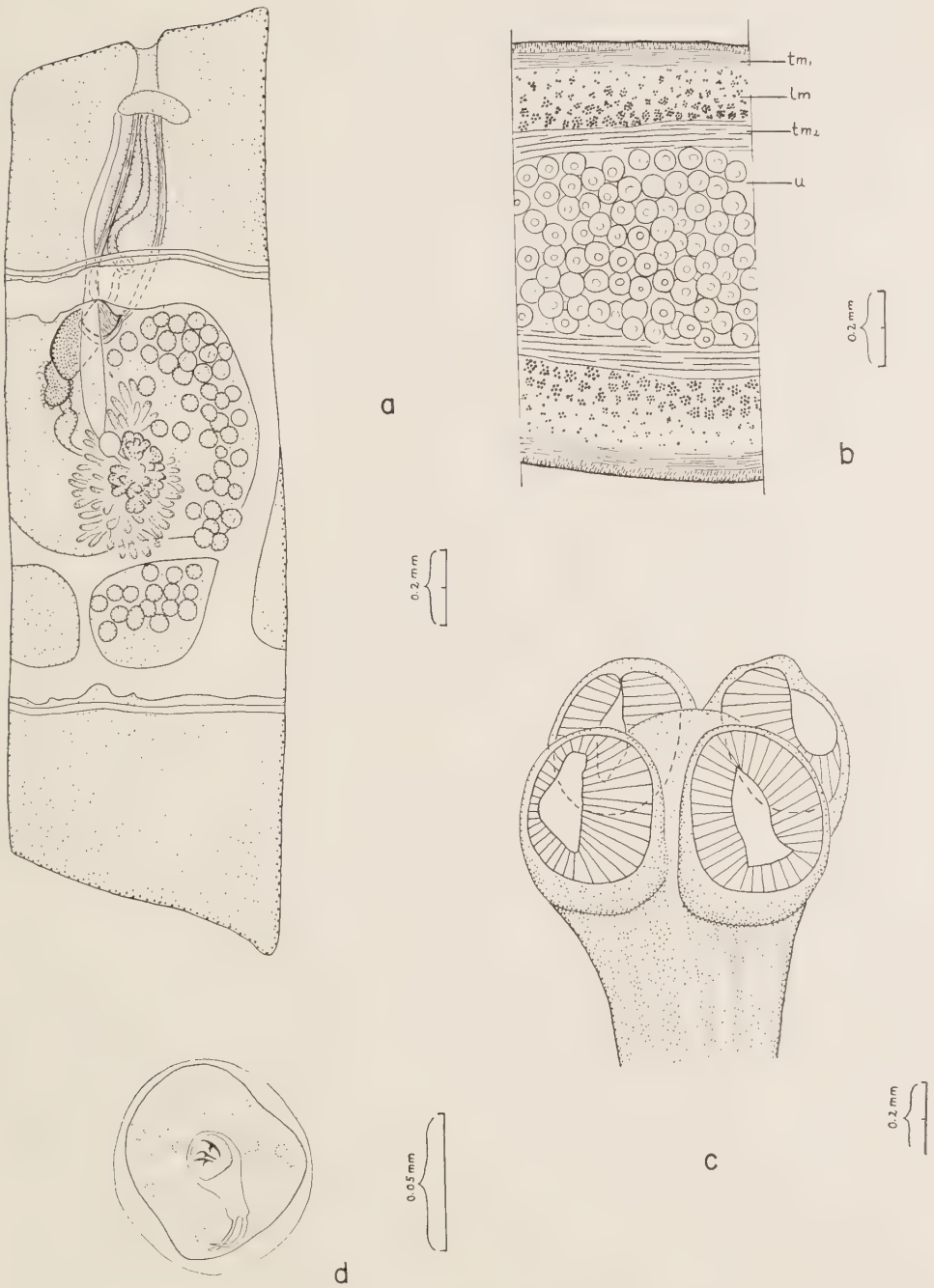

PLATE II

Monoecocestus rheiphilus

FIGS. a, Mature proglottid; b, Part of cross-section through gravid segment showing musculature and uterus filled with eggs; c, Scolex; d, Embryonated egg.

RESEARCH NOTES

SCAPHANOCEPHALUS EXPANSUS (CREPL.), A TREMATODE OF THE OSPREY, IN NORTH AMERICA

Examination of an osprey, *Pandion haliaetus carolinensis* furnished by Mr. Melvin Hoffman, Strawberry Point, Iowa, May, 1948, revealed about 30 trematodes which proved to be *Scaphanocephalus expansus* (Crepl.). This species which was redescribed in detail by Jägerskiöld, (1904; Results of the Swed. Zool. Exped. Egypt and White Nile 1901 pt. 1) has a very large "expanded" flap-like anterior end and, to my knowledge, resembles no other trematode in that respect. The specimens have been deposited in the U. S. Nat'l Mus. Helm. Coll. as No. 46437. Mr. Allen McIntosh (personal communication) has informed me that *S. expansus* has been recorded from the British Isles, Gulf of Suez, Egypt, and apparently other places in Europe and Asia. The present record extends its range to North America.

Several specimens of *Neodiplostomum sp.* were also recovered from the same osprey. It was impossible to identify these specifically because they had partially disintegrated. *Neodiplostomum tytense* Patwardhan, 1935, has been recorded from the osprey in India.

I wish to thank Allen McIntosh of the U. S. Bureau of Animal Industry and Dr. Ben Dawes of King's College, London, for assistance with host records and taxonomy.—GLENN L. HOFFMAN, *University of North Dakota.*

A NOTE ON *LYMNEA STAGNALIS* (L.) AS A SNAIL HOST FOR *FASCIOLOIDES MAGNA* (BASSI, 1875) (TREMATODA)

During an investigation to ascertain local intermediate snail hosts for the large American liver fluke (*F. magna*), laboratory bred *L. stagnalis* (L.) were experimentally infected. Snails were individually exposed in syracuse dishes to from four to six miracidia. One of ten young snails examined on the thirty-fifth day after exposure was found infected. A sporocyst with an actively moving mother redia was located on the wall of the respiratory chamber. Two young mother rediae were also found in the same vicinity. The parent snails used in this work were collected from Little Lake, Wellington County, Ontario, slightly north of the 43rd parallel of latitude.—L. Y. WU AND A. A. KINGSCOTE, *Department of Parasitology, Ontario Veterinary College, Guelph, Ontario, Canada.*

OBSERVATIONS ON EXCYSTATION OF METACERCARIAE OF *PLAGITURA SALAMANDRA* HOLL, 1928, WITH SOME NOTES ON ITS MORPHOLOGY

During the examination of four recently collected adult specimens of *Triturus viridescens,* a small number of metacercarial cysts, identified as *Plagitura salamandra* Holl, 1928, were found excysting in the intestinal lumen of one of the salamanders. Also present were 14 *Pseudosuccinea columella, 3 Gyraulus sp.,* 2 small dragon fly larvae, and 2 large damsel fly larvae in various stages of digestion. Each of these animals was isolated in physiological salt solution in an attempt to discover the source of the metacercariae. Upon dissection it was found that one *Gyraulus sp.* contained 53 living cysts, while the remaining snails and insects were uninfected.

The cysts were spheroidal to slightly ellipsoidal, thin walled, faintly orange in color, and with a slightly roughened surface. They were found primarily in the visceral mass of the snail, with only a few in the posterior portion of the foot. In size the cysts varied from 128 × 110 microns to 161 × 150 microns, a slightly greater range than that reported by Owen (1946, J. Parasit. 32: 553–557) in his description of the life history of this worm. While examining these cysts isolated in amphibian Ringer's solution, the process of excystation was observed 21 times. Initially a small clear spot was seen to form in the cyst wall at or near the anterior end of the worm, and then gradually increase in size and translucency. About ten minutes after the onset of the clearing, a small perforation appeared and soon increased in size until it was as large as the clear spot, which had reached a diameter of 60 to 70 microns. The worm, which had been quiescent or only sluggish, began to move vigorously as soon as the perforation appeared, and escaped when the opening became large enough. The entire process of excystation was accomplished in from 32 to 58 minutes by 18 of the individuals observed, but required more than four hours in the three other cases, one of which died in the process.

Measurements in microns of the stained mounted worms are as follows: body, 202–328 × 62–86; oral sucker, 29–39 × 31–42; acetabulum, 29–36; pharynx, 14–19; esophagus length, 42–100. These dimensions agree with those given by Owen (1946) except for the slightly larger range of body size, and the somewhat smaller size of both suckers.

These metacercariae differ from those of *Plagitura parva* Stunkard, 1933 by the larger body and cyst size, the nearly equal size of the suckers as compared to the relatively larger

acetabulum of *P. parva,* and the longer cornua of the excretory vesicle in *P. salamandra.* The method of excystation described above is also different from that described by Stunkard (1936, J. Parasit. 22: 354–374) for *P. parva,* in which the cyst wall splits on one side to permit escape of the worm.

It is of interest to note that these naturally occurring cysts were found in a new second intermediate host, *Gyraulus sp.,* but not in other snails and arthropods which not only occurred in the gut of four adult salamanders, but were successfully used as experimental intermediate hosts by Owen (1946). This information and the fact that the material used was collected in the type locality of *P. parva* suggest that natural infection of the second intermediate host of *P. salamandra,* and perhaps *P. parva,* depends on unknown ecological relationships.—Frank J. Etges, *Department of Biology, New York University.*

THE OCCURRENCE OF *TRITRICHOMONAS MURIS* (GRASSI) IN HETEROMYID AND CRICETID RODENTS OF CALIFORNIA

Kirby and Honigberg (1949; Univ. Calif. Publ. Zool. 53: 341) found *Tritrichomonas muris* (Grassi) in the caecum of *Dipodomys venustus.* This appears to be the only report of its occurrence in members of the family Heteromyidae. Kirby and Honigberg also found the flagellate in the cricetid rodents, *Peromyscus boylii, Peromyscus truei, Neotoma fuscipes* and *Microtus sp.* Kofoid and Swezy (1915; Proc. Amer. Acad. Arts Sci., 51: 289) reported it from *Peromyscus maniculatus gambelii* in California and Wenrich (1921; J. Morph., 36:119) reported that it occurred in *Peromyscus leucopus* in the eastern United States.

The writer has examined fresh and stained caecal contents from a number of rodents of the families Heteromyidae and Cricetidae. Flagellates similar in all respects to *Tritrichomonas muris* (Grassi) were found in the following hosts from the localities indicated: *Dipodomys agilis agilis* and *Peromyscus maniculatus gambelii* (Tujunga Wash, near San Fernando, Los Angeles Co.), *D. panamintinus morroensis* (near Morro Bay, San Luis Obsipo Co.), *D, nitratoides brevinasus* (McKittrick, Kern Co.), *Perognathus formosus mohavensis, Neotoma lepida* ssp., and *Perognathus longimembris longimembris* (Lovejoy Butte, near Palmdale, Los, Angeles Co.), *Perognathus parvus* ssp. (Jackass Spring, Inyo Co.) and *Peromycus californicus* ssp. (Santa Monica Canyon, Los Angeles Co.)—David J. Doran, *University of California, Los Angeles.* (Present Address: Bureau of Animal Industry, Beltsville, Maryland.)

SURVIVAL TIME OF METACYCLIC *TRYPANOSOMA CRUZI* IN HUMAN SWEAT

Human contaminative contacts with bug feces containing *Trypanosoma cruzi* Chagas are most likely during the warmer months in California since adult *Triatoma protacta* invade human habitations from June to September. Much of the inland area under 4000 feet is very warm and dry in summer with high temperatures persisting through the night to early morning. Therefore, it would seem logical that if man were contaminated by *Triatoma* feces at this time of year, there would be some sweat on the skin. What effect would this have on active metacyclic *Trypanosoma cruzi?*

Naturally and experimentally infected adult *Triatoma protracta* with long-standing infections of O'Neals *Trypanosoma cruzi* were isolated in wide mouth specimen jars after feeding on a guinea pig. Various voluntary fecal samples were selected from the first through the seventh droppings for any one bug at any one time. The control group consisted of 3 females and 2 males from which seven samples were watched under an 18 mm. circular coverglass until motility ceased in all trypanosomes. The mixtures were approximately equal parts of clear bug feces (single droplets) and sodium citrate solution transferred to slides with a fine glass pipette. Four of the samples were rimmed with paraffin and three were not. The average survival time of metacyclic trypanosomes as judged by motility for the seven samples was 76.7 minutes, the extremes being 20 minutes (paraffin) and 157 minutes (no paraffin) at laboratory temperatures between 32° and 34° C. at the time of observation. The experimental group consisted of 3 females and 3 males from which twelve samples were watched. These mixtures were approximately equal parts of citrated trypanosomes (proportions the same as the control group) and sweat from the writer's epigastric fossa. In two samples the proportion was 2/3 citrated trypanosomes and 1/3 human sweat. Four samples were rimmed with paraffin, eight were not. The average survival time of trypanosomes for the twelve samples was 17.1 minutes, the extremes being 10 (paraffin) and 33 minutes (no paraffin) at laboratory temperatures between 32° and 32.5° C. at the time of observation. Apparently, fresh human sweat mixed with sodium citrate solution immobilizes *Trypanosoma cruzi* Chagas.

The writer wishes to thank the California Forest and Range Experiment Station and the Department of Zoology at Davis, University of California, for use of facilities at the San

Joaquin Experimental Range, O'Neals, California.—Sherwin F. Wood, *Los Angeles City College, Los Angeles 29, California.*

EPIZOOTIC IN EASTERN OREGON MUSKRATS ASSOCIATED WITH MASSIVE INFECTION OF *HYMENOLEPIS ONDATRAE* RIDER AND MACY

During the winter of 1947 a large number of muskrats, *Ondatra zibethicus,* at Malheur Lake National Wildlife Refuge at Burns, Oregon, died from an undiagnosed malady. Several iced carcasses were sent to the senior author for examination. A thorough check of the organs revealed that the small intestine of these animals contained thousands of *Hymenolepis,* so many, in fact, that occlusion of the lumen was a distinct possibility. Examination of muskrats of the same group by a competent state bacteriology laboratory did not reveal the presence of any recognized bacterial or virus disease, which strongly suggests that the cestodes could have caused the epizootic. Confirmation was not possible since further material could not be shipped and lack of time prevented the undertaking of a trip to Malheur Lake for further investigation.

Special thanks are due to several persons who have helped in this study, especially Mr. J. C. Scharff, and Mr. Leo L. Laythe, Regional Director of the U. S. Fish and Wildlife Service. This work was supported by Contract No. 153(00) between the Office of Naval Research, Department of the Navy, and Reed College, Portland, Oregon.

A recent careful study of the *Hymenolepis* demonstrated it to be *H. ondatrae* Rider and Macy (1947; Trans. Amer. Micro. Soc., 66:176–181;) although some slight differences are noted. The slightly shorter length can be attributed to crowding. Size and form of the rostellar hooks are the same as in the Western Oregon material although the number of these structures was consistently eight, whereas in the original description it was reported as varying from eight to ten. This suggests that the Eastern Oregon material represents a population differing in some degree from the type specimens, but in the writer's opinion the peculiarity is not sufficient for the creation of a new name at present. Another variation noted involved the uterus, which in Eastern Oregon specimens fills the greater part of the ripe proglottides extending nearly to the lateral margins and overlapping the cirrus pouch. In the more anterior of the gravid segments it has a trilobed appearance; however, in the terminal segments the uterus apparently is not lobed. This condition is different from that of the type material in which the uterus is trilobed to the terminal segments. No empty proglottides were observed in the Malheur Lake specimens.

Reporting the epizootic, Mr. J. C. Scharff, superintendent of the refuge, stated: "During the last month (January–February) to six weeks there has been a considerable die-off of muskrats . . . from no apparent cause. At first this die-off was noticed only over a small area, but later it seems to have spread to most of the 'rat-inhabited waters. As many as fourteen dead 'rats have been found in one house."—Ralph W. Macy and June Anderson Biggs, *Reed College, Portland, Oregon.*

FURTHER STUDIES ON THE OCCURRENCE OF THE DOG HEART WORM, *DIROFILARIA IMMITIS* IN DOGS IN MISSISSIPPI

The heart worm of dogs, *Dirofilaria immitis,* has long been known to occur in dogs of the south and southwest. Bird hunters and fox hunters know that this parasite greatly reduces the stamina of hunting dogs. It would also appear that the presence of this parasite, in quantity, would alter the results obtained from experiments dealing with cardiovascular physiology.

The most abundant intermediate hosts of *Dirofilaria immitis* in Mississippi are the rain barrel mosquito, *Culex quinquefasciatus,* and a tree hole breeding mosquito, *Aedes triseriatus.*

Ward and Reeder (Jour. of Parasitology, vol. 34, Sec. 2, 1947) reported the results obtained from the examinations of 31 dogs. Direct examination of 10 dogs revealed that 5, or 50 percent, were infected with the adult heart worm. Examination of the peripheral blood of three dogs revealed that 1, or 33 percent, demonstrated microfilaria in the blood, and the examination of the serum of 18 dogs, revealed that 1, or 5.56 percent of that group had microfilaria in the blood.

During a six month period, February through July, 1951, the writers made a study of the internal parasites of 153 dogs. The animals were contributed by the department of physiology after they had been sacrificed at the completion of experiments by students or by the staff. The greater number of the animals were adults. The hearts of these animals were examined by incising the right ventricle. When present the worms were usually entwined around the chordae tendinae which attach the cusps of the tricuspid valve to the papillary muscles and

to the walls of the right ventricle. In case of heavy infection, the parasites extended in to the pulmonary arteries.

Fifty of the 153 dogs, or slightly over 30 percent, were infected with the heart worm. The number of worms recovered from individual dogs ranged from 1 to 38. More of the larger breeds of dogs were found to be infected than were the smaller breeds. More short haired dogs were infected than were long haired animals. The percentage of infection was greater in dogs examined during the months of March and April.—J. W. Ward and M. A. Franklin, *University of Mississippi, School of Medicine.*

FIRST REPORT OF THE HUMAN INTESTINAL FLUKE *HETEROPHYES HETEROPHYES* FROM A YEMEN BAT, *RHINOLOPHUS CLIVOSUS ACROTIS*

Through the courtesy of Don Heyneman of the Department of Biology, Rice Institute, I have been enabled to examine a rather extensive collection of slides and preserved trematodes of bats from Yemen and Egypt obtained by Robert E. Kunz and Harry Hoogstraal of the United States Naval Medical Research Unit at Cairo, Egypt. (see *National Geographic Magazine,* 101 (Feb.): 213-244, 1952). Among these were two small but mature specimens of the human intestinal fluke *Heterophyes heterophyes* (von Siebold, 1852), from *Rhinolophus clivosus acrotis* taken on February 14, 1951, at San'a, Yemen. This is the first record of the species in a bat, as far as the writer is aware, although it occurs in such carnivores as cats, dogs and foxes.

Measurements, in millimeters, of the two specimens from the bat are as follows: Body length 0.63 and 0.90; maximum body width 0.31 and 0.36; width of oral sucker 0.063 and 0.067, length 0.044 and 0.061; diameter of ventral sucker 0.10 and 0.16; width of genital sucker 0.095 and 0.12; width of pharynx 0.038 and 0.044, length 0.044 and 0.059; diameter of testes about 0.08 and 0.09; diameter of ovary 0.04 and 0.06; diameter of seminal receptacle 0.05 and 0.08; eggs in the uterus 0.012–0.015 wide by 0.023–0.025 long. The latter are slightly smaller in dimensions than figures given by Looss (Centralbl. Bakt. Parasit., 1894) who stated that the eggs measure 0.017 by 0.030. These figures agree with those given by Craig and Faust (*Clinical Parasitology,* Philadelphia, ed. 5, p. 532, 1951) who listed them as 0.015 to 0.017 by 0.028–0.030. Body length is 2 mm. according to Looss and 1–1.7 mm. as given by Craig and Faust, and the body width is stated by the same authors to be 1 mm. and 0.3–0.4 mm. respectively. Integumentary scales are as described for the species, but appear not to extend quite as far caudad as claimed by others. However, they reach at least as far back as the level of the seminal receptacle. Multidigitate spines form a crown on the muscular genital sucker and correspond in arrangement to previous descriptions. The slide bearing the two specimens from the bat has been deposited in the U. S. National Museum.

The presence of this fluke in a bat at once raises the question as to how this mammal could obtain the fish which serves as second intermediate host. Bats commonly fly close to the surface of water in twilight at a time when small fish are jumping and quite possibly might scoop some into their mouths. Allen (*Bats,* Cambridge, Mass., pp. 93, 138, 299. 1939) stated that a number of species of Chiroptera sometimes catch fish but no mention is made of the habit in the bat involved here.

This work was supported by Contract No. 153(00), Nr 132-992, between the Office of Naval Research, Department of the Navy, and Reed College, Portland, Oregon.—Ralph W. Macy, *Reed College, Portland, Oregon.*

AN OBSERVATION ON THE LOSS OF MICROFILARIAE FROM THE MOSQUITO HOST DURING ITS INFECTIVE MEAL

Anopheles quadrimaculatus has the habit of expelling one or more droplets of blood-tinged plasma from the anus during satiation feeding. The nature of the dejecta is apparently due to a significant separation of plasma and corpuscles in the midgut (Boyd, 1949, Malariology, V. 1, pp. 608–697). Since this phenomenon was a regularly observed occurrence during recent studies with *Dirofilaria immitis* in the mosquito host (Kartman, 1953, Exper. Parasit. 2: 27–78) it was decided to determine what filarial loss, if any, resulted.

Thirty-one *A. quadrimaculatus* females were confined individually to small glass feeding jars the ends of which were covered with black bobbinet of a texture which did not absorb the drop of anal exudate released by the mosquito. As the female fed to repletion on an infected dog, a large droplet usually was expelled from the anus and this was immediately drawn up with a fine capillary pipette containing a small amount of normal saline. This mixture was placed on a glass slide and examined under the microscope. A record was kept of all microfilariae found in the droplets and, likewise, the number of microfilariae in the same mosquito's midgut.

The number of microfilariae found per anal droplet varied from zero to 8 and the loss of parasites per mosquito varied from zero to 17 per cent. Of a total of 1599 microfilariae counted from the 31 female mosquitoes used, 1487 parasites were found in the midgets whereas 112 or 7 per cent were found in the dejecta. No such anal dejecta were observed when *Aedes aegypti, A. albopictus, Culex quinquefasciatus,* and *C. pipiens* fed on a dog infected with *D. immitis.*

Mitzmain (1917, Pub. Health Rep. *32*: 1400–1413) noted that gametocytes of human malaria are eliminated by various species of *Anopheles* and indicated that the mosquito ejects several droplets during the first twenty-four hours after feeding. If this situation is true for *A. quadrimaculatus* it may be possible that greater numbers of parasites actually are lost than have been indicated here. However, this would depend upon how soon after feeding the droplets, subsequent to the initial one, are ejected since microfilarial migration out of the midgut is quite rapid in this host species. The loss of parasites by anal exudate is probably of no quantitative significance to the host efficiency of *A. quadrimaculatus* since it has been shown to be a very favorable host for *D. immitis* (Kartman, *ibid*).—L. KARTMAN, *The Johns Hopkins University, School of Hygiene & Public Health.* Present address, U. S. Public Health Service, Honokaa, Hawaii, T. H.

EFFECT OF FEEDING MOSQUITOES UPON DOGS WITH DIFFERENTIAL MICROFILARAEMIAS

It may be reasonable to assume that the rate of filarial development in the mosquito host is affected by the number of microfilariae which successfully reach tissues favoring their growth. Some evidence bearing on this problem was obtained from records of the rate of larval development of *Dirofilaria immitis* in *Anopheles quadrimaculatus.*

Four lots of female mosquitoes were fed on dog S with from 16,000 to 18,000 microfilariae per cm^3, and three lots were fed on dog D with from 30,000 to 34,000 parasites per cm^3. All feedings and estimates of microfilariaemia were done at 2:30 P.M. The infected mosquitoes were dissected fifteen days after the infective blood meal and all larval filariae in them recorded These data are given in Table 1.

TABLE 1.—*Larval development in* Anopheles quadrimaculatus *of* Dirofilaria immitis *from two different hosts**

Lot #	No. ♀♀	Dog	Mlpg. tubules	Number of larvae of each stage recovered after 15 days: Third stage in: Abd.	Thx.	Head	Labium	Per cent: 2nd stage	3rd stage
1	10	S	19(2nd) 1(3rd)	9	12	89	10	13.5	86.5
2	10	S	30(2nd) 20(3rd)	5	14	39	18	23.8	76.2
3	10	D	896(2nd) 12(3rd)	1	0	3	0	98.2	1.8
4	10	S	132(2nd) 72(3rd)	13	22	19	5	50.1	49.9
5	10	D	1075(2nd) 2(3rd)	0	0	0	0	99.8	0.2
6	10	S	13(2nd) 15(3rd)	6	40	63	28	7.8	92.2
7	10	D	983(2nd) 6(3rd)	2	0	0	0	99.2	0.8

* All tests in air conditioned insectary at approximately 27° C. and 85–90 per cent Rel. Hum.

It should be noted that the 40 females fed on dog S had an average of 17.3 parasites each, whereas the 30 females fed on dog D averaged 99.3 filarial larvae each. Furthermore, of a total of 105 females originally fed on dog S, 50 or 47.6% survived for fifteen days, whereas 5% or 45 of 900 females fed on dog D survived during the same period.

The obvious question is whether these differences are due to the considerable variation in numbers of microfilariae ingested by the mosquitoes from the two hosts, or whether strain heterogeneity in the parasite may account for the phenomenon. Since dog S was obtained from St. Augustine, Florida and dog D from Sarasota, Florida the argument for strain variance in the parasite seems less appealing than the suggestion that the differences in rate of larval development and survival of the mosquitoes were due to the disparity in numbers of parasites ingested by the mosquitoes.—L. KARTMAN, *The Johns Hopkins University, School of Hygiene & Public Health, Baltimore.* Present address, U. S. Public Health Service, Honokaa, Hawaii, T. H.

INGESTION BY MOSQUITOES OF SALINE AND SUGAR SUSPENSIONS OF *DIROFILARIA IMMITIS* MICROFILARIAE

During the course of experiments with the dog heartworm some preliminary observations were made on the ingestion by mosquitoes of microfilariae in liquids other than blood. Microfilariae of *Dirofilaria immitis* were isolated free of blood elements by the method of Franks and Stoll (1945, J. Parasit. 31: 158–162) with slight modifications. Microfilariae were suspended in physiological saline (0.85 per cent) and offered to females of *Aedes aegypti* and *Culex pipiens* by means of the artificial feeding apparatus designed by Greenberg (1949, Mosquito News, 9: 48–50). None of the *Culex* individuals fed and only 4 of 75 *A. aegypti* accepted the saline despite the fact that these mosquitoes had been deprived of food and water during the previous 24 hour period. In contrast, *A. aegypti* will readily ingest erythrocytes of various animals and man suspended in saline and will ingest saline solutions containing as little as one-sixteenth to one-thirty-second original volume of erythrocytes (Greenberg, 1951, J. Nutrition, 43: 27–35). One of the engorged *A. aegypti* was dissected one hour after feeding and its midgut was found to be distended with saline and many active microfilariae could be seen moving in the stomach. The dorsal and ventral diverticula were filled with gas bubbles and appeared completely devoid of liquid or microfilariae. Another female was dissected one day later and proved negative for filariae. A third female, dissected two days after feeding, contained 4 "sausage" stage larvae in its Malpighian tubules. The fourth female was dissected nine days after its saline feeding and its tubules contained 2 microfilaria-like larvae and 3 developing larvae in the pre-infective (2nd) stage.

In another test *D. immitis* microfilariae were suspended in a solution containing 49½ grams of dextrose in 1 liter of distilled water. The microfilariae in this solution appeared normal after thirty minutes when observed under the microscope. This sugar solution with microfilariae was offered to both sexes of *Anopheles quadrimaculatus* in the Greenberg apparatus and about 25 females and 5 males fed upon it; the males fed from drops placed upon the netting enclosing them in a lantern chimney. Female *A. quadrimaculatus* controls were fed on infected dog whole blood which had undergone centrifugation similar to that employed when microfilariae were isolated from the blood. Dissection of three females about one hour after feeding on the sugar solution showed empty midguts but distended diverticula containing active microfilariae. Seventeen days later 15 female and 2 male *A. quadrimaculatus* were dissected; all of these were completely negative for filariae. On the other hand, all of 12 control females showed normal development of the worms to the infective stage during the same period. These results suggest that microfilariae which entered the diverticula failed to find their way to the midgut and thus into the Malpighian tubules. These results appear somewhat unexpected since even conflicting views on the distribution of liquids in the diverticula and stomach agree that sugary solutions find their way to the midgut either by later passage from the diverticula or during ingestion when small amounts may go directly to the stomach (Trembley, 1952, Amer. J. Trop. Med. Hyg. 1: 693–710).—L. KARTMAN, *The Johns Hopkins University, School of Hygiene and Public Health.* Present address, U. S. Public Health Service, Hawaiian Field Station, Honokaa, Hawaii, T. H.

FERTILIZATION OF THE CESTODE *SCHISTOCEPHALUS SOLIDUS IN VITRO*

In a series of papers over the past six years, it has been shown that plerocercoids of *Schistocephalus solidus* and *Ligula intestinalis* may be cultured to maturity *in vitro* provided culture conditions are carefully controlled. These results have, however, been marred by the fact that only infertile eggs are produced *in vitro*. That fertilization does not normally take place *in vitro* is readily demonstrated by the fact that in sections of cultured worms the *receptaculum seminis* in each proglottid is devoid of spermatozoa, whereas in normal worms (matured in the bird gut) the receptaculum is always filled with spermatozoa (Smyth, 1950, J. Parasit. 36: 371–383.)

It has long been suspected that failure of fertilization was due to lack of compression during maturation *in vitro*. Considerable technical difficulties arise when a compression is attempted, the most important being the accumulation of acidic metabolic waste products which rapidly kill the worms. After developing and rejecting a number of techniques, these difficulties have now been overcome by means of a quarter-inch seamless cellulose tubing, which acts as an admirable artificial gut, compressing larvae during cultivation and, at the same time being semi-permeable, permitting the escape by diffusion of metabolic waste products. The medium is constantly agitated to assist this diffusion.

Two to four larvae of *Schistocephalus* are cultured within a length of the cellulose tubing (Visking Corporation, 6733 W. 65th St., Chicago, 38, Ill.)—itself suspended in a tube of horse serum; the whole tube is placed in a shaker in a water bath at 40° C. and shaken continuously during the period of incubation (2 days); sterile procedure is maintained throughout.

Eggs produced from worms matured under these conditions showed a fertility as high as 77% and hatched apparently normal coracidia. Sections of cultured worms showed receptacula filled with spermatozoa, thus indicating that fertilization had taken place. This technique, which may need further elaboration, is one which is likely to be applicable to other parasitic organisms.

The author is indebted to the Medical Research Council of Ireland for financial aid, and to Mr. Desmond Fitzgerald for first obtaining samples of the cellulose tubing.—J. D. SMYTH, *Trinity College, Dublin, Ireland.*

ECHINOCOCCUS INFECTION IN MISSISSIPPI. A NEW RECORD OF A NATURAL INFECTION IN DOGS

During the spring and summer of 1951, while making a survey of the helminth parasites of dogs, one of us (M. A. F.) encountered two very heavy infections of the adult of *Echinococcus granulosus.* Since both dogs were from the same locality it might be assumed that the parasite is endemic in that area. A total of 50 dogs has been examined from the area. So far as the writers have been able to ascertain, this is the first report of this parasite occurring in Mississippi. In recent years there have been several reports of cysts of *Echinococcus* in hogs and cattle from Virginia, Oklahoma, Arkansas, and Louisiana. The hydatid is not uncommon in moose, and to a lesser extent, in deer in Minnesota. The adult is also common in wolves from the same area (Riley; 1933 Minn. Med. 16: 744–745). It appears that there are three records of dogs having been found to be naturally infected with *Echinococcus granulosus.* Curtice, (1892; J. Comp. Med. Vet. Arch. 13: 223–236) reported a natural infection from a dog in Washington, D. C. McCune, (1935; J. Amer. Vet. Assn. 36: 210–211) reported a similar infection in a dog from Tacoma, Washington. This animal was a guard dog of a rabbitry in which one rabbit was found to harbor the hydatid of the parasite in the intercostal muscles. The third case was reported by Crowe, (1907; Johns Hopkins Hosp. Bull. 18: 464–467). The dogs which harbored the parasites were obtained from an area of Mississippi where many transients are imported as cotton pickers. The majority of them reside in South Texas and Mexico. Also in this area are a number of dogs that have spent some time in training in Northern Minnesota and Canada. Sheep have also been imported from a number of areas of the United States.—M. A. FRANKLIN, AND J. W. WARD, *University of Mississippi, School of Medicine.*

FORMATION IN THE RABBIT OF ANTIBODIES AGAINST *NIPPOSTRONGYLUS MURIS*

When rats are infected with *Nippostrongylus muris,* antibodies are formed in the animal against the secretions and excretions of the worms which precipitate these products *in vitro* as described by Sarles (1938, J. Infect. Dis. 62: 337). It has been shown that rabbits can be infected with this worm (Thorson, J. Parasit. 39:575) but the percentage development of the worms is low and the infection is of short duration. It was considered desirable to test the sera of rabbits which had been repeatedly infected with living larvae of *N. muris* for the presence of antibodies which could precipitate secretions and excretions of the worms *in vitro.* Two rabbits received 8500, 47,000, 66,000, 132,000, 200,000, and 200,000 larvae at 5 day intervals. The feces of these animals contained eggs of *N. muris* from the sixth to the tenth days after the initial infection, but none were seen after this time. Four days after the last infection, the animals were bled by cardiac puncture. The serum was separated and stored. Larvae of *N. muris* were placed on microscopic slides (on which paraffin rings had been made) in normal rat serum, immune rat serum, normal rabbit serum and immune rabbit serum. Antibiotics were added to reduce bacterial growth and the preparations were covered with sterile cover slips. Precipitates, both oral and excretory, appeared in the immune rat serum and in the immune rabbit serum in 1 hour. No precipitates occurred on larvae in either normal rat serum or normal rabbit serum. However, in two days the larvae in both normal and immune rabbit sera were almost 100 per cent dead whereas those in normal and immune rat sera were alive for the week of observation.—RALPH E. THORSON, *School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.*

THE LENGTH OF THE PREPATENT PERIOD IN A FILARIAL INFECTION OF DUCKS

During a 3-year study of the epizootiology and the phenomenon of relapse in *Leucocytozoon simondi* infections of ducks (Am. J. Hyg. 56:39–57, 101–118, 1952), groups of hatchery-bred White Pekin ducklings were exposed to natural infection on farms in the vicinity of the University of Michigan Biological Station, Cheboygan, Michigan. Birds that contracted and

survived *L. simondi* infections were shipped to Baltimore at the conclusion of each summer (1948–49–50) and their infections were followed by means of thin films taken 3 times weekly during the ensuing winter and spring months; in 1948–49, each film was examined microscopically under high-dry magnification for 15 minutes, while during the following 2 years blood films were each examined for 5 minutes. Each winter, usually during the months of December–February, microfilariae (species not determined) appeared in the peripheral blood of some of the birds that had acquired *L. simondi* infections during the preceding summer. By the time the project was concluded in the spring of 1951, the onset of microfilaremia had been observed in a total of 16 such birds. Since the literature is almost devoid of information pertaining to the duration of the prepatent period in filarial infections, the pertinent data for the 16 cases are presented in this note.

Although the 16 ducklings are treated here as a single group, 5 came from among those exposed during the summer of 1948, 6 from the series of 1949, and 5 from the 1950 series. All had been hatched in May of the year they came under study and all were purchased from the same commercial source at Alma, Michigan. They were shipped from the hatchery to Cheboygan by rail and were then protected from insect bites prior to farm exposure and again after removal from the farms. The periods of exposure ranged from 8 to 12 days (during July) in the case of 3 ducklings, and 2 months (July and August) in the remaining 13 birds. The 16 ducks became microfilaria-positive as follows: 4 in December, 5 in January, 6 in February, and 1 in March. Dating from the beginning of the exposure periods to the first appearance of microfilariae, the prepatent period ranged in length from 6 to 9 months with a mean of 7.2 months. Six of the ducks were examined at necropsy for adult filariids but none were found despite painstaking effort.

It does not seem at all likely that any of these birds could have been infected while still in the hatchery at Alma, although this possibility cannot be excluded with certainty. However, even allowing for this, the error in estimating the length of the prepatent period cannot be more than 6 weeks since the ducklings were of that age when received from the hatchery. A further source of possible error rests in the fact that the data are based solely on thin-film examinations while more appropriate methods might have disclosed microfilaremia somewhat earlier.—Eli Chernin, *School of Hygiene and Public Health, The Johns Hopkins University, and the University of Michigan Biological Station.* (Present address: Harvard School of Public Health, Boston).

INFECTION OF RABBITS WITH A RAT NEMATODE, *NIPPOSTRONGYLUS MURIS*

Abnormal host-parasite relationships in nematode infections have been studied quite extensively with *Nippostrongylus muris,* [Porter (1935, J. Parasit. 21: 314; 1935, Amer. J. Hyg. 22: 444) and Lindquist (1950, Am. J. Hyg. 52: 22.)], but no records have been found of attempts to infect rabbits with this rat nematode. Eight six-week old rabbits were injected subcutaneously with various doses of larvae of *N. muris.* Feces were collected on the sixth to the twelfth days. Two of the rabbits that had received 2000 larvae had eggs in their feces on days 6–9. These eggs were cultured in bone charcoal and in 5–7 days larvae appeared on the surface of the cultures. They were removed from the charcoal and injected into 4-week old rats and eight days later at autopsy these rats had large numbers of adults of *N. muris* in their intestines. The other 6 rabbits received from 3000–25,000 larva and eggs were present in their feces from the seventh to the ninth days. None were found after this period. Eight days after infection, the rabbit which had received 25,000 larvae was autopsied and 2 adult males of *N. muris* were found in the intestine. Two additional rabbits were infected with graded doses of larvae over a period of a month and their feces contained eggs of *N. muris* from the sixth to the tenth days after the first injection.

There was a low percentage development of *N. muris* in the rabbit, the duration of infection was short, but it is of interest that some worms developed to adulthood in this host.—Ralph E. Thorson, *School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.*

ERRATA

Vol. 39, No. 4 (1) August 1953, p. 431, for line 3 of table, substitute: "Nov. 1949, after harvesting from stock cultures on 7 Oct."

Vol. 39, No. 3 June 1953, p. 260, line 6 of paragraph 2 should be line 4 of paragraph 4.

ANNOUNCEMENT

At the meeting of the American Society of Parasitologists, held at Madison, Wisconsin, September 7, 8, 9, 1953, Doctor George R. LaRue was elected Chairman of the Editorial Committee of the Journal of Parasitology for the five-year term 1954–1958. Since material to complete Volume 39 (1953) is now in galley, all manuscripts hereafter submitted for publication in the Journal of Parasitology should be addressed to Doctor George R. LaRue, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Md.